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On the Early Development of the Vestimentiferan Tube Worm *Ridgeia* sp. and Observations on the Nervous System and Trophosome of *Ridgeia* sp. and *Riftia pachyptila*

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Abstract. Stages in the development of the vestimentiferan *Ridgeia* sp., based on lengths of preserved specimens processed for scanning electron microscopy, were examined. Features of the nervous system and trophosome of *Riftia pachyptila* were studied by light, scanning-, and transmission-electron microscopy. Development proceeds from a trochophore-type larva with an anterior prototrochal ciliary ring and a posterior assemblage of transient larval setae, through intermediate stages, some of which lack endosymbiotic bacteria but all of which display additional transient features such as larval branchial filaments, a ventral medial process, and digestive tract, to a young juvenile stage that possesses endosymbiotic bacteria and exhibits the morphology characteristic of adult vestimentiferans. Larval branchial filaments are resorbed in later developmental stages and replaced by paired rows of ciliated branchial filaments with pinnules. The larval gut is divisible into foregut, midgut, and hindgut regions based on cytological features of the epithelium. The establishment of the symbiotic association in the midgut region is confirmed. The development of the gut and the establishment of the endosymbiotic association appear to be correlated with the timing of settlement by the young juveniles. Aspects of the development of the nervous system include the appearance of the brain near the base of the ventral medial process followed by the development of a nerve cord in the epithelium of the body wall throughout the length of the juvenile. The nerve cord includes one or two giant axons, except in its most posterior region. The trochophore larva likely serves as a dispersal stage in the life history of vestimentiferans. The trochophore larva in the early development of vestimentiferans strengthens the assertion that Vestimentifera and Annelida are closely related.

Introduction

The first vestimentiferan species, *Lamellibrachia barhami*, was described by Webb (1969) from a cold-water site in the eastern Pacific. Among other features, the absence of a gut led Webb to place this species in the phylum Pogonophora. In their description of a second vestimentiferan species, *L. luymesi*, van der Land and Nørrevang (1975) introduced the term "trophosome" for a peculiar tissue that occupies the trunk region. In a later report, van der Land and Nørrevang (1977) examined in greater detail the organization of the "parenchymatous" trophosomal tissue and stated that it consists of numerous small lobules. In each lobule, a peripheral layer of pigment cells surrounds a central region of basophilic cells filled with numerous vacuoles. Van der Land and Nørrevang suggested that the trophosome functions as a liver and is also a source of nutrients for developing spermatozoa. In the same year, Webb (1977) described and illustrated a "spongy tissue" associated with the male reproductive system of *L. barhami*, but he did not suggest a function for this tissue nor did he refer to it as trophosome. In his description of a third vestimentiferan species, *Riftia pachyptila*, Jones (1981a) noted the presence of trophosomal tissue in the trunk region. In a subsequent report, Jones (1981b) described the trophosome as consisting of many lobules, each with a central blood vessel that gives rise to numerous capillaries that ramify throughout the tissue. In their description of a third species of *Lamellibrachia*, *L. victori*, Mañé-Garzón and Montero (1986) commented on the lobular nature of the trophosome and its vascularization. Further, they stated, apparently on the basis of light microscopy, that there

are ". . . numerous accumulations of symbiotic microorganisms which are not bacteria but spores or algae" (p. 18). These observations have not been confirmed by other investigations.

Based on transmission electron microscopic (TEM) observations and analysis of lipopolysaccharide, Cavanaugh (1980) suggested that the bulk of the trophosome of *R. pachyptila* is occupied by bacteria that potentially serve as chemoautotrophic symbionts. In the first study of trophosomal tissue to include TEM micrographs, Cavanaugh *et al.* (1981) confirmed the presence of bacteria in the trophosome of *R. pachyptila*. Subsequent studies have confirmed the presence of endosymbiotic bacteria in *R. pachyptila* (Bosch and Grassé, 1984a, b; Hand, 1987), *Escarpia spicata* (Felbeck *et al.*, 1981; Cavanaugh, 1983a, b; reported as an unnamed new species), *Lamellibrachia barhami* (Felbeck *et al.*, 1981), *Ridgeia piscesae, Ridgeia phaeophiale,* and two additional undescribed species of vestimentiferans (de Burgh, 1986), *Oasisia alvinae* and *Ridgeia* sp. (the latter two species from personal TEM observations by SLG). These and other studies have also provided insight into the nature of the relationship between the bacteria and their hosts (for recent references, see Felbeck and Childress, 1988). One question that arises with respect to this association is how it is established in new individuals.

Jones (1985b, 1987, 1988b) and Jones and Gardiner (1988) noted the presence of a so-called ventral medial process (=siphon; Southward, 1988b) at the base of the branchial plume of juvenile *R. pachyptila, Ridgeia* sp., and *Oasisia alvinae*. The ciliated aperture at the distal end of the process leads to a duct that passes through the brain, through the vestimentum, and communicates with the established trophosome. They suggested that the aperture and duct are the means of entry of bacteria into the trophosome. Jones (1987) suggested that free-living bacteria are collected at random by the ciliated aperture of the process and transported to the trophosome via the duct, and if such bacteria include sulfide-oxidizing bacteria and others necessary for the worm, the worm survives; if they do not, the worm ultimately dies. Jones and Gardiner (1988) stated that the ciliated aperture at the distal end of the ventral medial process is the mouth. In addition, they described the gut and anus of a complete digestive tract in *Ridgeia,* as well as the fine structure of the foregut of *Riftia* and of cell junctions of its trophosomal bacteriocytes. Southward (1988a, b) also reported on the digestive tract in early developmental stages of *Ridgeia,* suggested an early phase of ciliary feeding, and discussed the relationship of the Vestimentifera and Pogonophora and the relationship of these to the Annelida.

Preliminary results of additional studies of early developmental stages of *Ridgeia* were presented by Jones (1988a) and form the basis of expanded results presented in this report, which includes the first description of a trochophore larva in the development of vestimentiferans. Additional new information is provided on the development of the ventral process and digestive system, the nervous system, larval and opisthosomal setae, branchial filaments, ventral ciliated field, and vestimentum. Observations on certain aspects of the development of the trophosome are presented. When appropriate, results from Southward (1988b) are compared with our findings.

Materials and Methods

Larval and juvenile specimens of *Ridgeia* sp. were collected at "Axial Seamount," Juan de Fuca Ridge (*Alvin* Dive 1413, 45°56′N; 130°01′W, 18 July 1984, 1546 m depth, and *Alvin* Dive 1924, 45°55′N; 130°02′W, 30 September 1987, 1540 m depth). Specimens of *Riftia pachyptila* were collected on the Galapagos Rift at the "Rose Garden" hydrothermal vent site (*Alvin* Dive 889, 00°48.7′N; 86°12.7′W, 14 February 1979, 2458 m depth) and a juvenile *Riftia* was collected at the "Garden of Eden" vent site (*Alvin* Dive 993, 00°47′N; 86°08′W, 10 December 1979, 2518 m depth). Additional specimens of *Riftia pachyptila* were obtained at the "Clam Acres" site on the EPR at 21° N (*Alvin* Dive 1225, 20°50′N; 109°06′W, 9 May 1982, 2618 m depth). For all specimens, except those from "Clam Acres" and the 1987 collections from "Axial Seamount," after initial fixation in 10% formalin (buffered with $CaCO_3$, pH 6.95) in seawater, the specimens were transferred to 70% ethanol.

For light microscopy, specimens were dehydrated and embedded in a mixture of epon-araldite, using propylene oxide as the infiltration solvent; semi-thin sections (1.5 or 2.0 μm) were cut on a Sorvall MT-2 ultramicrotome and were stained with Masson's triple stain. For scanning electron microscopy (SEM), specimens were placed in Ruthenium Red (as a mordant for OsO_4) for one hour, post-fixed in 1% OsO_4, on ice, for one hour, dehydrated through a graded ethanol series, and then critical-point or freeze dried. Specimens were sputter-coated with gold-palladium (about 15-nm thick) and examined in either a Cambridge 100 Stereoscan or a Hitachi S-570 scanning electron microscope. The collections from "Clam Acres" were intended for examination by transmission electron microscopy; samples of adult trophosome were fixed aboard ship at room temperature in 3.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.3) containing 10% sucrose and a trace of $CaCl_2$. Tissues were refrigerated and stored in glutaraldehyde fixative until their use for this study. The recent collections from "Axial Seamount" (1987) yielded *Ridgeia* larvae/juveniles for light microscopy and TEM examination from a clump of tubes of *R. piscesae,* bulk-fixed onboard ship in approximately 4% glutaraldehyde in 0.1 *M* cacodylate buffer (pH 7.2). Tis-

sues for TEM were ("Clam Acres") post-fixed in phosphate-buffered OsO_4 for 1 h at 4°C and dehydrated in a standard ethanol series, or ("Axial Seamount") post-fixed in cacodylate-buffered OsO_4 for 2 h at room temperature, pre-embedded in agar, and dehydrated in a graded series of ethanol, with 2,2-dimethyoxypropane after 70% ethanol. Trophosome was embedded in a mixture of epon-araldite and larvae/juveniles in agar blocks were embedded in Spurr's resin; propylene oxide was the infiltration solvent in both procedures. Thin-sections were cut on a Sorvall MT-2 ultramicrotome, stained with aqueous uranyl acetate and lead citrate, and examined in a JEOL 100S, JEM 1200EX, or a Zeiss EM9S-2 transmission electron microscope.

Measurements of length of the 38 specimens of *Ridgeia* examined by SEM are not "total length" (Table I). Due to the variability of contraction of branchial filaments of preserved specimens, as well as the lack of branchial filaments in early stages, lengths were measured from the prototrochal band of larvae and the base of branchial filaments of juveniles to the posterior extremity. This length, of specimens processed for SEM, is the basis for the ranking of specimens and does not necessarily reflect a true estimate of actual or relative age of the specimens. Where appropriate, rank numbers (#X) are included in the text and figure legends to identify individual specimens listed in Table I. Those illustrated specimens not ranked are noted as "nr. #X" to indicate their length relative to ranked specimens. The lengths of sectioned specimens were adjusted to take into account shrinkage due to processing for SEM. A comparison of measurements before and after critical-point drying indicated a shortening of body length by about 7.5% during processing; this factor was applied to lengths of sectioned material to make them comparable to SEM specimens. This shrinkage was variable (the 7.5%, above, is an average of shrinkage of specimens ranging from 4% to 10%), and may explain apparent inconsistencies in some of the results below. Where a comparison with the observations of Southward (1988b) was appropriate, measurements, from the base of branchial filaments to the posterior end, were based on her SEM micrograph and camera lucida drawings, using her indicated scales and correcting for shrinkage, and were converted to "nr. #'s" as follows:

Fig. 3A,—0.727 mm—nr. #27
Fig. 4, left—0.119 mm—nr. #6
Fig. 4, right—0.108 mm—nr. #5
Fig. 5A, spec. RJ—0.142 mm—nr. #9
Fig. 5C, spec. RB—1.304 mm—nr. #32
Fig. 5D, spec. RC—1.613 mm—nr. #33

Two species of *Ridgeia* are known from Axial Seamount and, although the 38 larvae and juveniles examined by SEM were sorted from clumps of *Ridgeia piscesae* tubes, it is not possible to determine their specific identity. On the basis of overall body shape, specimens with rank numbers 6, 7, 17, and 19 may be different from the rest of the series; rank numbers 15 and 22 have an unusually thick ventral process; these characteristics may be artifacts of fixation or processing.

The Vestimentiferan Body Plan and the Systematic Relationships of the Vestimentifera

The Vestimentifera presently contains ten species whose anatomy and morphology have been described to varying degrees (see Jones, 1985c; Mañé-Garzón and Montero, 1986). Although differences exist pertaining to certain aspects of the anatomy and morphology of these ten species, they, nevertheless, share a common body plan as adults. The following description, based largely on observations from Jones (1985c), is intended to familiarize the reader with this vestimentiferan body plan. It is our desire that subsequent sections of this report, which describe certain aspects of the development of *Ridgeia* sp. and the anatomy of *Riftia pachyptila,* can be examined with the characteristic adult body plan of the Vestimentifera in mind.

The body of juvenile and adult vestimentiferans is divisible externally into four distinct regions. From anterior to posterior ends, these regions are (1) the obturacular region, (2) the vestimentum, (3) the trunk, and (4) the opisthosome (Fig. 1).

The obturacular region (Fig. 1, OB) comprises the central obturaculum (Fig. 1, OBT), which supports and bears the respiratory plume, and the plume itself; the latter consists of many branchial filaments (Fig. 1, BF) that are provided with pinnules (lobes that increase the respiratory surface) and are fused as left and right series of branchial lamellae. In the case of *R. pachyptila,* the branchial lamellae extend perpendicularly from the obturaculum and are free for most of their length (class Axonobranchia). In all other vestimentiferans described to date (class Basibranchia), the branchial lamellae extend anteriorly from the base of the obturaculum and are fused for most of their length. One or two openings of the excretory organ are situated on the dorsal surface near the base of the obturaculum.

The vestimentum (Fig. 1, VS) is provided ventrally with a conspicuous pear- or teardrop-shaped ciliated field (Fig. 1, VC), which is bounded by the two parts of the separated ventral nerve cord. Plaques (Fig. 9, PL) and the openings of the so-called pyriform glands are also evident on the ventral and lateral surfaces; the latter secrete tube material at and near the open end of the tube. Dorsally, the vestimentum bears a pair of genital apertures with paired ciliated grooves extending anteriorly in the

Table I

Rankings (RK) of specimens of Ridgeia *sp. observed by scanning electron microscopy (SEM), arranged by length (L = distance from prototroch or base of branchial filaments to posterior end), and notations of presence of larval setae (LS), number of branchial filaments (BF), condition of ventral ciliated field (VCF), number of rows of opisthosomal setae (OS), state of vestimentum (VST), obturaculum (OBT) and anus (AN) and presence or absence of endosymbiotic bacteria (BA)*

RK		L (mm)	LS	BF	VCF	OS	VST	OBT	AN	BA
1		0.058	P	0	L	0	L	L	?	?
2		0.075	P	0	L	0	L	L	?	?
3		0.087	P	2	S	?	L	L	?	?
4		0.100	P	3	S	1	L	L	?	?
	*G	0.104 (0.112)	P	2	S	0	L	L	L	L
5		0.110	?	2	S	?	L	L	?	?
6		0.111	P	2	L	0	L	L	?	?
7		0.140	P	2	S	1	L	L	?P	?
8		0.141	P	3	S	1	L	L	?	?
9		0.143	P	2	S	1	L	L	P	?
	*F	0.148 (0.160)	P	2	S	1	L	L	L	L
10		0.150	P	3	S	1	L	L	P	?
11		0.155	P	3	S	1	L	L	?	?
12		0.160	P	5	S	1	L	L	?	?
13		0.162	P	2	S	1	L	L	P	?
	*D	0.163 (0.176)	?	2	S	1	L	L	L	L
14		0.169	P	2	S	1	L	L	?	?
	*H	0.176 (0.190)	P	4	S	1	L	L	P	P
	*B	0.178 (0.192)	?	4	S	1	L	L	P	L
15		0.180	P	5	S	1	L	L	?	?
16		0.180	P	4	S	2	D	L	?	?
	*E	0.182 (0.197)	P	2(?+)	?	1	L	L	L	L
17		0.184	P	2	S	1	L	L	L	?
18		0.190	P	4	S	1	L	L	?	?
19		0.214	P	2	?	1	L	L	?	?
20		0.220	?	5	S	2	D	L	?	?
	*CB7	0.247 (0.267)	?	5	?S	1	L	L	?	P
		0.257	P	?4	S	1	L	L	L	?
	*C	0.272 (0.294)	?	4	S	1	L	L	P	L
21		0.280	P	2	S	2	D	L	?	?
	*A	0.285 (0.308)	?	6	?S	1	L	L	P	P
22		0.300	P	5	S	2	L	L	?	?
23		0.400	P	4	?	2	P	L	?	?
	*CB1	0.400 (0.432)	?	8	?	3	P	L	L	P
24		0.440	P	M	F	1	P	L	?	?
25		0.540	P	8	F	2	P	L	?	?
26		0.690	L	M	F	4	P	?	?	?
27		0.750	P	M	F	4	P	?	?	?
28		1.010	P	M	F	7	P	P	?	?
29		1.060	L	M	F	?	P	?	?	?
30		1.120	?	M	F	8	P	?	?L	?
31		1.210	P	M	F	5	P	P	?	?
32		1.340	P	M	F	9	P	?	?	?
33		1.560	P	M	F	8	P	P	?	?
34		1.740	P	M	F	7	P	P	L	?
35		2.000	L	M	F	4	P	?	?	?
36		2.190	L	M	F	7	P	P	?	?
37		2.230	L	M	F	11	P	P	L	?
38		4.040	L	M	F	22	P	P	L	?

Lengths of sectioned specimens, marked with "*" and an identifying letter, are adjusted to allow for lack of shrinkage due to SEM processing and actual lengths follow, in parentheses. D: developing; F: fused; L: lacking; M: many, not countable accurately; P: present; S: segmented; ?: not known, unobservable.

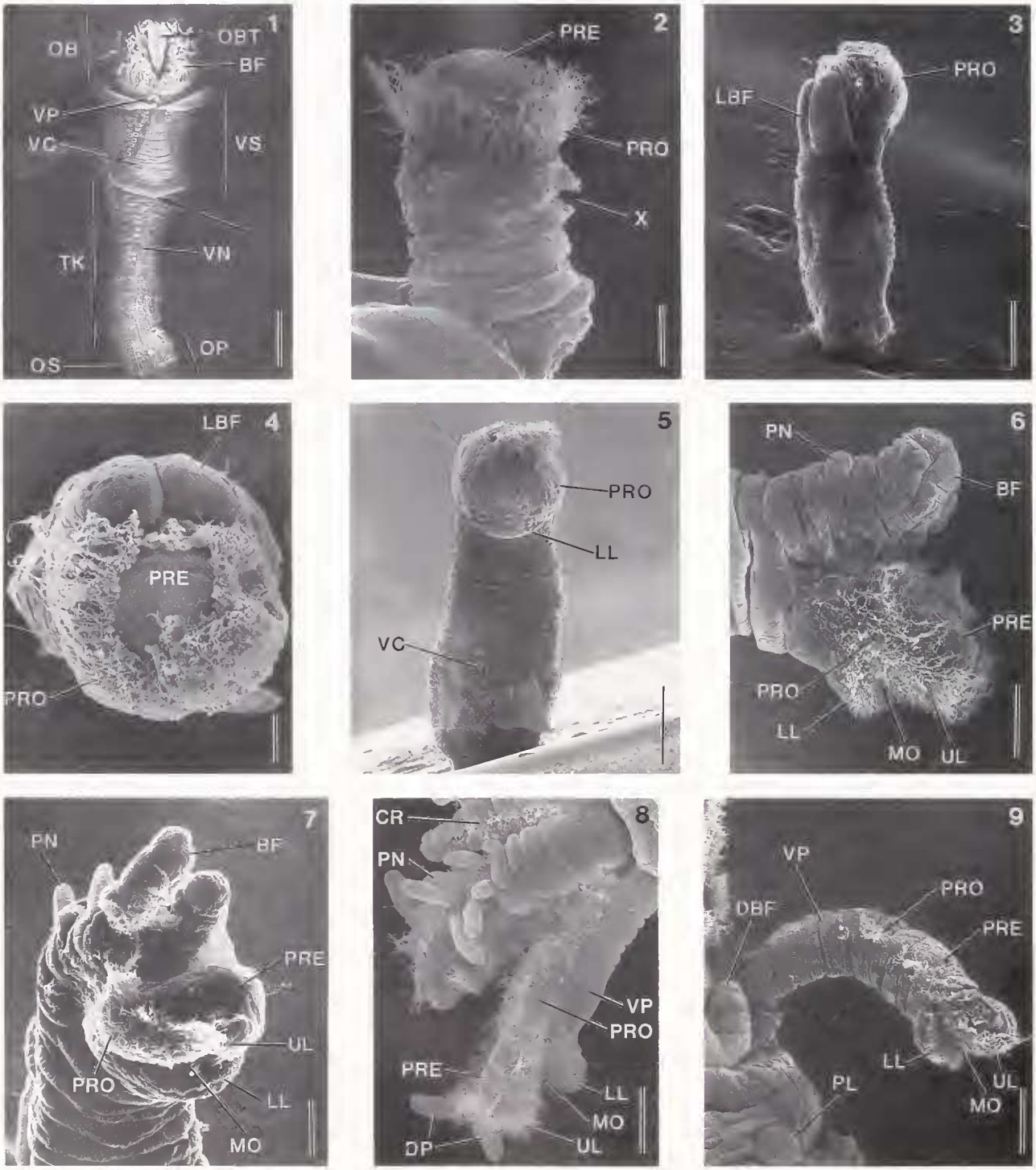


Figure 1. *Ridgeia* sp. Rank #36, SEM. Ventral view, juvenile. Arrow, posterior margin of vestimentum. Scale bar, 500 μm.

Figure 2. Rank #1, SEM. Trochophore larva. Scale bar, 15 μm.

Figure 3. Rank #3, SEM. Right dorsolateral view of larva. Scale bar, 30 μm.

Figure 4. Rank #3, SEM. Anterior view of larva of Fig. 3. Scale bar, 10 μm.

Figure 5. Rank #3, SEM. Ventral view of larva of Fig. 3. Scale bar, 30 μm.

Figure 6. Rank #5, SEM. Right lateral view, ventral process of larva. Scale bar, 20 μm.

Figure 7. Rank #14, SEM. Anterior view, anterior region of larva, developing ventral process; tip of branchial filament damaged. Scale bar, 20 μm.

case of males. Internally, the bulk of the vestimentum consists of muscles and connective tissue and these act to keep the vestimentum at the open end of the tube and maintain the position of the branchial plume of the obturacular region outside the tube; the brain is situated ventrally near the anterior margin and the excretory organ is posterior to the brain.

The trunk region (Fig. 1, TK) occupies the greatest relative portion of the body in all vestimentiferans so far described (up to 80% of total body length in the largest specimen of *R. pachyptila*; Jones, 1981a). The surface of the trunk bears the trace of the united nerve cord in the ventral midline (Fig. 1, VN) and the papillae of pyriform gland openings; secretions from the latter thicken the tube wall. Internally, the trunk contains vascular elements, the trophosome, which houses endosymbiotic bacteria, and the gonad. A digestive tract has not been observed in this region in adult specimens.

The opisthosome (Fig. 1, OP) is the only region of the body that is multisegmented in the adult. Externally, the continued trace of the nerve cord is visible in the ventral midline. Anterior segments are provided with transverse rows of opisthosomal setae (Fig. 1, OS) that act as a holdfast to the inner surface of the tube when the worm withdraws; a variable number of posterior segments lack setae but are indicated externally by furrows that mark the positions of internal septa. Internally, the coelomic space of each segment is paired due to the presence of a median mesentery that extends throughout the length of the opisthosome.

The systematic relationship of the Vestimentifera with other higher Bilateria is still unsettled. This lack of agreement among investigators centers in large part on the importance of the segmentation pattern (and the arrangement of coelomic spaces) and the manner of segment formation in the Vestimentifera relative to other segmented groups. Webb (1969) cited the long trunk and opisthosome (=metasoma) of *Lamellibrachia barhami* as distinctive pogonophoran features and placed that species in a new class and order in the phylum Pogonophora. Van der Land and Nørrevang (1975, 1977) did not attribute special phylogenetic importance to the regionation of the body of *Lamellibrachia* and considered the Vestimentifera, as well as the Pogonophora, as separate classes in the phylum Annelida. In his original description of *Riftia pachyptila,* Jones (1981a) considered the body regionation as indicating a close relationship with the pogonophorans. He retained the Pogonophora at the level of phylum and placed the Vestimentifera in it as a new subphylum, the Obturata. Jones (1985a) contrasted the arrangement of the apparent segments of the vestimentiferans and pogonophorans and the development of segments in the opisthosome. He suggested that these two groups may not be as closely related as previously thought and that their close relationship with the Annelida required reexamination. Based on previous observations and additional new information, Jones (1985b, c) separated the Vestimentifera and Pogonophora at the level of phyla. Citing, in particular, an apparent lack of difference in the development of segments in the opisthosomes of the vestimentiferans and pogonophorans, Southward (1988b) suggested that the two groups should be considered as subclasses in the class Pogonophora. In addition, she suggested that the outcome of future developmental studies would be instrumental in determining if the class Pogonophora should be placed in the phylum Annelida or phylum Brachiata.

Results

Trochophore

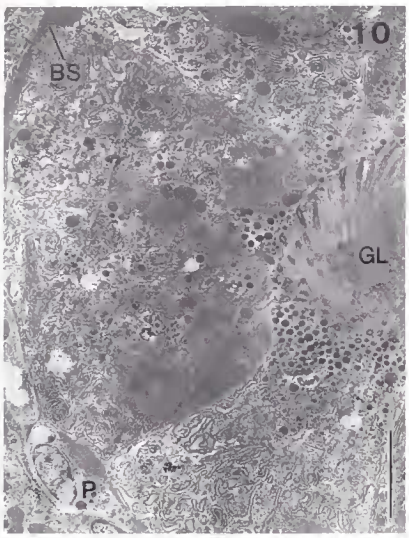
The trochophore larva of *Ridgeia* is provided with a prototrochal ring of cilia (Fig. 2, PRO) and lacks a neurotroch, metatroch, and an apical tuft on the pretrochal area (Fig. 2, PRE); that part of the posterior region that is not obscured by mounting adhesive does not reveal a telotroch (Fig. 2). Examination of the prototroch at higher magnification does not reveal the presence of compound cilia; this may be due to a less than optimal fixation of the original sample or may reflect the true state of the prototrochal cilia. There are no apparent mouth or anal openings. That the specimen is a vestimentiferan trochophore is confirmed by the presence of larval setae typical of later larval and juvenile stages (see Discussion, below).

Ventral process and gut

At about the time that the first pair of larval branchial filaments develop (Figs. 3, 4, LBF) (see Branchial filaments, below), a lip-like protrusion (Fig. 5, LL) arises just posterior to the prototroch (Figs. 3–5, PRO). This, in ad-

Figure 8. Rank #15, SEM. Left lateral view, ventral process of larva. Scale bar, 30 μm.

Figure 9. Rank #25, SEM. Right lateral view, ventral process of juvenile. Scale bar, 30 μm. BF, branchial filament(s); CR, ciliary row; DBF, developing branchial filament; DP, displaced pinnules; LBF, larval branchial filaments; LL, lower lip; MO, mouth; OB, obturacular region; OBT, obturaculum; OP, opisthosome; OS, opisthosomal setae; PL, vestimental plaque; PN, pinnule; PRE, pretrochal region; PRO, prototroch; TK, trunk; UL, upper lip; VC, ventral ciliated field; VN, ventral nerve cord; VP, ventral process; VS, vestimentum; X, damage during processing.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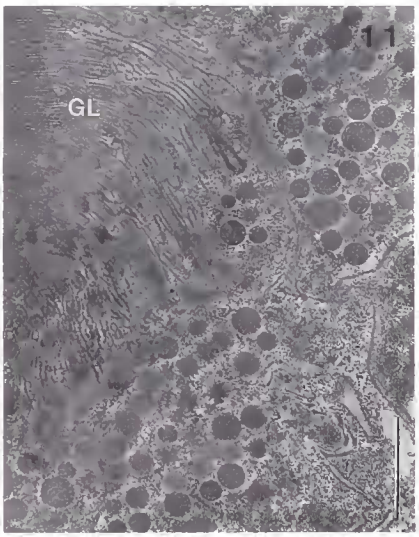
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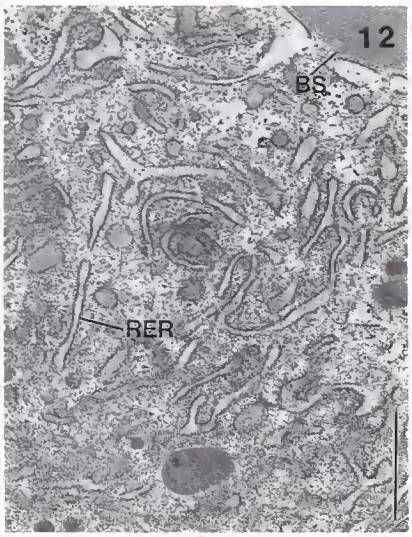
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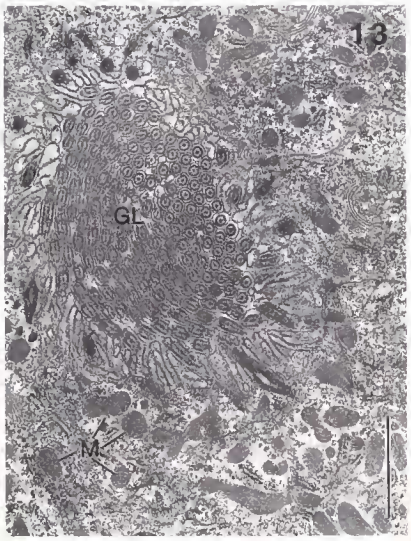
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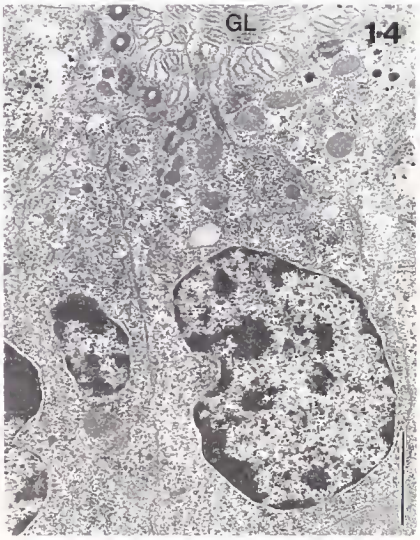
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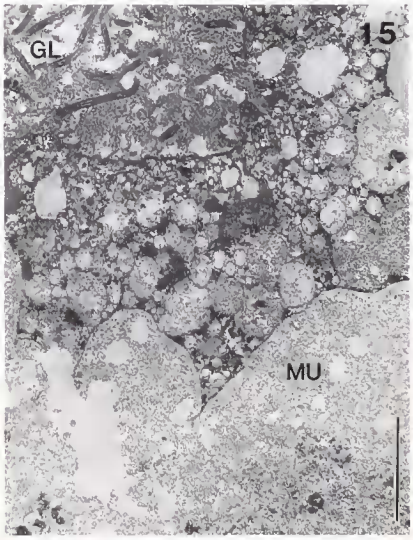
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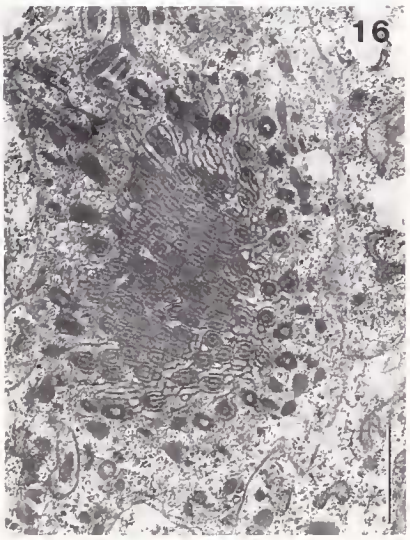
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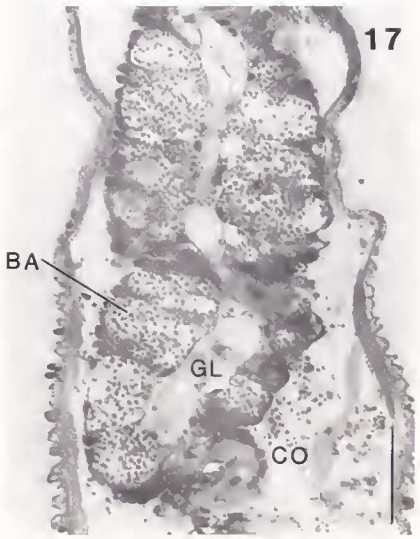
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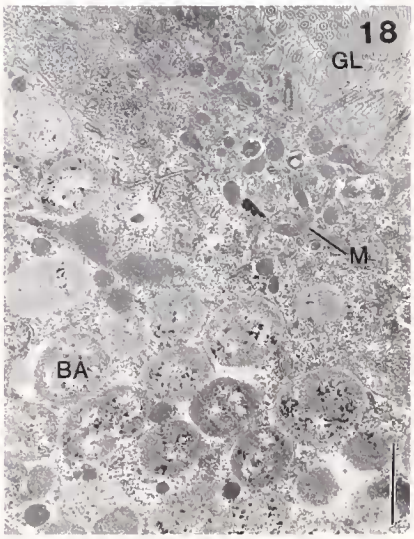
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dition to the first appearance of the ciliated field (Fig. 5, VC), establishes the ventral surface of the developing larva. As in the case of the trochophore, close examination of the prototroch failed to reveal the presence of compound cilia. In time, the posttrochal protrusion develops cilia and becomes the lower (=posterior) lip of the mouth opening (Figs. 6, 7, LL). The mouth is formed between the lower lip and the ventral portion of the prototroch; the latter becomes the upper (=anterior) lip of the mouth (Figs. 6, 7, UL). There is a differential growth such that the prototroch is displaced ventrally and the ventral process, with its terminal mouth, is formed (Figs. 6, 7, MO). The residuum of the prototroch is distributed laterally along the length of the process (Fig. 8, PRO) and persists for some time as the process elongates (Fig. 9, rank #25, PRO). The entire pretrochal region of the trochophore appears to be restricted to the upper (=anterior) surface of the ventral process (Figs. 6–9, PRE).

Examination of sectioned specimens by light microscopy and whole specimens by SEM (see Table I) reveals details of several aspects of the early development of the gut of *Ridgeia*. First, the mouth is formed early in development (G-nr. #4) but is not connected to the foregut (F-nr. #10, D-nr. #13, E-nr. #16) until later in development (H-nr. #15, CB7-nr. #20, with endosymbionts; C-nr. #21, lacking endosymbionts). Second, the anal opening is established after the mouth is open, but the stage at which the anus appears, and a complete digestive system can be established, is variable. Third, the appearance of the anus seems to precede the establishment of the bacterial association in young juvenile stages (note specimens B-nr. #15 and C-nr. #21 in Table I). Fourth, the bacterial association is established in the midgut region of *Ridgeia*, but the time at which this association occurs is variable (in particular, note specimens H- and B-nr. #15, C- and A-nr. #21). Finally, the closure of an anus in later juvenile stages, *e.g.*, #34, #37, and #38, is correlated with the probability that the symbiotic association with bacteria has been established (see Discussion, below).

Throughout its length, the gut is lined by an epithelium of multiciliated cells whose cilia nearly obscure the lumen (Figs. 10, 13, 16). The presence of accessory centrioles and a system of rootlets associated with basal bodies of the cilia have not been confirmed. When viewed by TEM, a foregut, midgut, and hindgut are distinguishable in *Ridgeia*, based on cytological features of the epithelial cells.

The foregut epithelium is characterized by the presence of numerous electron-dense secretory granules up to 500 nm in diameter in the apical region of the cells (Figs. 10, 11). Mitochondria are scattered in the cytoplasm beneath the area occupied by the granules. Basally, the cells contain extensive profiles of rough endoplasmic reticulum (Fig. 12, RER) and numerous Golgi complexes that are actively releasing vesicles. The foregut epithelium rests on a blood sinus (Figs. 10, 12, BS), and a layer of peritoneal cells is situated between this sinus and the body wall (Fig. 10, P).

Prior to the establishment of the symbiotic association, the cytology of the epithelial cells of the midgut is rather unremarkable. Apically, the cells contain numerous mitochondria, scattered Golgi complexes, and a few electron-dense granules (Fig. 13). Nuclei of the cells are situated basally (Fig. 14) and RER is not extensively developed. A thin layer of extracellular matrix separates the epithelial cells from a layer of peritoneal cells. A blood sinus was not observed in our specimens.

When viewed by light microscopy, the hindgut appears transparent when compared with other regions of the gut (Figs. 21, 45, 49, 50). This observation is accounted for in TEM preparations in that the hindgut epithelial cells contain large vacuoles that are filled with a slightly granular, electron-translucent substance, which may represent mucus or unused yolk material (Fig. 15,

Figure 10. *Ridgeia* sp. Unranked juvenile, TEM. Cross-section of foregut epithelium. Note secretory granules in region of cells adjacent to gut lumen. Scale bar, 4 μm.

Figure 11. Unranked juvenile, TEM. Enlargement of apical region of foregut epithelium, showing numerous secretory granules. Scale bar, 1.5 μm.

Figure 12. Unranked juvenile, TEM. Enlargement of basal region of foregut epithelium, showing rough endoplasmic reticulum. Scale bar, 1.5 μm.

Figure 13. Unranked juvenile, TEM. Apical region of midgut epithelium prior to establishment of bacterial association. Scale bar, 2 μm.

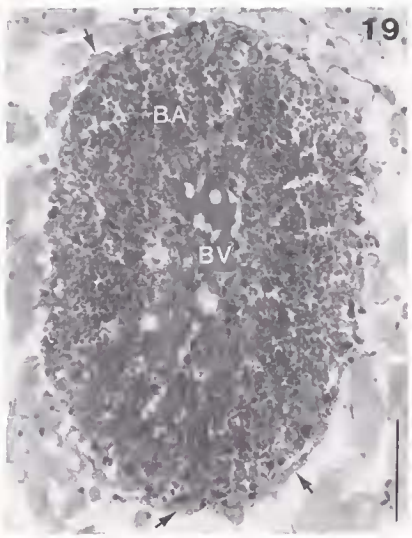
Figure 14. Unranked juvenile, TEM. Cross-section of midgut epithelial cell prior to establishment of bacterial association. Scale bar, 2 μm.

Figure 15. Unranked juvenile, TEM. Hindgut epithelium. Scale bar, 3 μm.

Figure 16. Unranked juvenile, TEM. Tangential section of epithelium surrounding anal opening. Scale bar, 1.5 μm.

Figure 17. Rank A-nr. #21, frontal section, epon, 2.0 μm. Midgut of juvenile. Scale bar, 30 μm.

Figure 18. Unranked juvenile, TEM. Cross-section of midgut epithelium after establishment of bacterial association. Scale bar, 2 μm. BA, bacteria; BS, blood sinus; CO, coelom of trunk; GL, lumen of gut; M, mitochondria; MU, mucus; P, peritoneum; RER, rough endoplasmic reticulum.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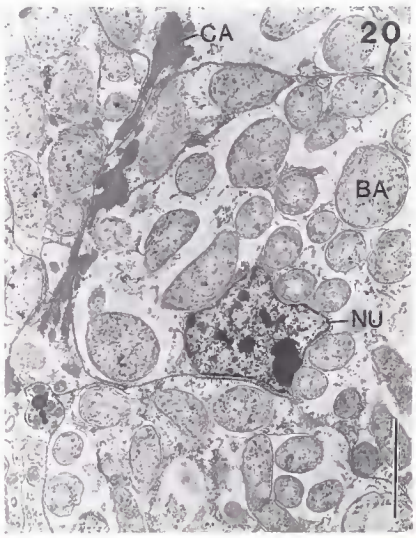
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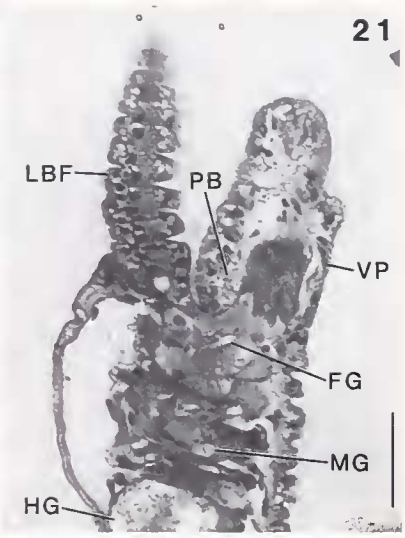
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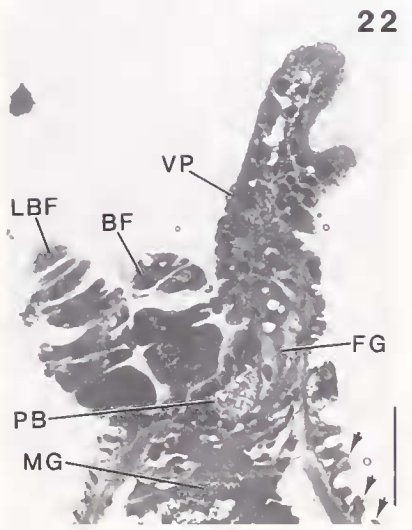
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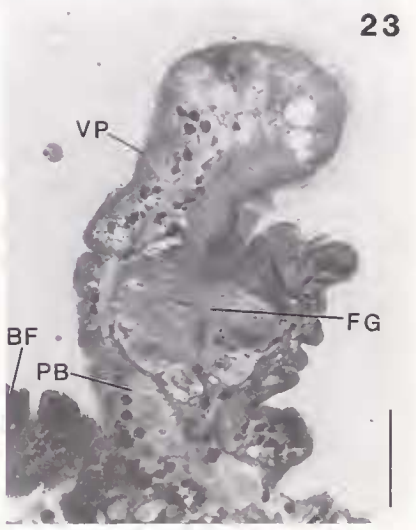
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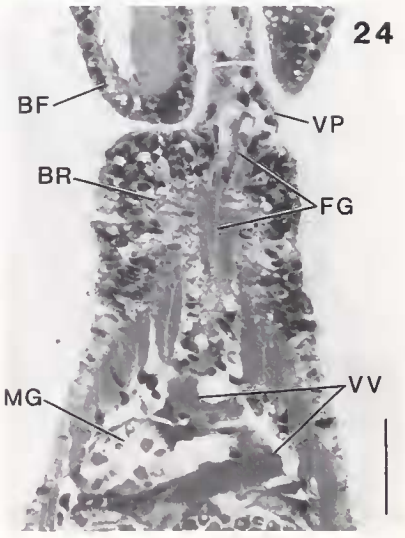
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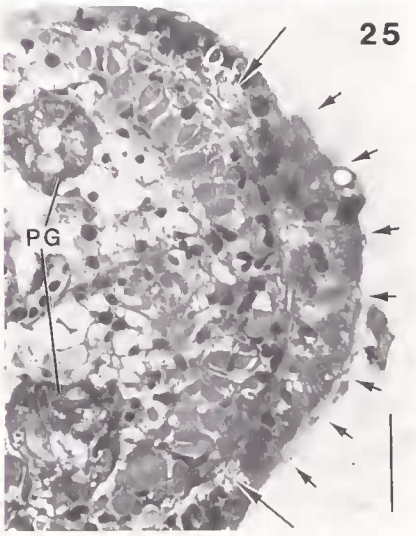
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MU). Cytoplasm is restricted to a thin layer around the periphery of the cells, adjacent to the membranes. Scattered mitochondria are present in the apical region of the cells, but nuclei and other cellular organelles have not been observed. Bacteria and other materials are present in the lumen of the hindgut. An associated layer of peritoneal cells appears to be absent. In the region of the anus, the cytology of the epithelial cells resembles that of the midgut (Fig. 16).

Bacterial association and trophosome

The bacterial association is established in the midgut region of *Ridgeia*. During early stages of the association, the lumen of the gut is open, and the bacteria appear to be scattered throughout the epithelial cells (Figs. 17, BA, GL). However, when viewed by TEM, the bacteria (Fig. 18, BA) are seen to occupy the basal area of the epithelial cells. Bacteria are coccoid in shape, up to 2.5 μm in diameter, and appear to be housed in separate vacuoles. Nuclei of the epithelial cells and mitochondria are situated in the cytoplasm surrounding the bacteria (Figs. 18, M; 20, NU). The presence of different sizes of bacteria or of the digestion of bacteria by midgut cells, as noted by Southward (1988b), were not confirmed in our specimens. At least one instance of a bacterium undergoing binary fission has been observed (pers. obs., SLG). Mitochondria, RER, and, occasionally, a Golgi complex, are observed in the apical region of the cells (Fig. 18).

As development proceeds, the lumen of the midgut disappears, and the trophosome develops through an elaboration of the original epithelial lining of the midgut. In adults of *Riftia pachyptila,* and presumably all vestimentiferans, the trophosome consists of numerous elongated lobules that appear circular or somewhat elliptical when viewed in cross-section (Fig. 19). Each lobule is provided with a central axial blood vessel from which extend numerous capillaries 1.0–3.6 μm in diameter that connect with blood vessels on the outer surface of the lobule (Fig. 19, arrows; for additional details on the vascular system of the trophosome, see Jones, 1988b). The cytology of the specialized cells that house the bacteria in the trophosome (bacteriocytes) differs from that of the original midgut epithelium mainly in the absence of most cellular organelles. Only the nucleus and a few scattered mitochondria have been observed in the cytoplasm surrounding the bacteria (Fig. 20). Analysis of TEM micrographs indicates that bacteria occupy at least 40% of the area of a bacteriocyte, in section, and that bacteriocytes account for at least 41% to 53% of the total area of the trophosome, in section.

Nervous system

In the development of the ventral process, accumulations of presumed nervous tissue are present at the base of the process (Figs. 21–23, PB), just below its dorsal (=anterior) surface. These arise between the outer layer of epithelial cells and the wall of the foregut. There are suggestions of continuity of this nervous tissue, lateral to the foregut and to just internal to the ventral surface; this growth around the foregut has yet to be confirmed at these stages. The presumptive brain, within the ventral process, has been observed in stages D-nr. #13, H-nr. #15 and C-nr. #21 (Figs. 21–23, PB), but not in presumed younger stages (G-nr. #4 and F-nr. #10). Later, the brain comes to be situated in the vestimentum, internal to the ventral process, posterior to the branchial filaments, with the foregut traversing it (Fig. 24, BR, FG).

In larval and young juvenile stages (up to C-nr. #21), a differentiated ventral nerve cord has not been observed in the epithelium of the body wall. In later juvenile stages and adults, a single nerve cord exits the brain on its ventral surface, just internal to the cuticle and epithelium of the body wall (see Jones, 1981a, 1985a, for additional descriptions of the nervous system of adult vestimentiferans). In addition to other nervous tissue, the nerve cord, here, contains a pair of giant axons. In juveniles of *Ridgeia* sp. and *Oasisia alvinae,* and, presumably, all vestimentiferans, the perikarya of these giant axons are situated adjacent to each other in the dorsal region of the brain. In contrast to other cells in the brain, the cyto-

Figure 19. *Riftia pachyptila* USNM No. 59958, adult, transverse section, paraffin, 5 μm. Cross-section of lobule of trophosome. Arrows, surface blood vessels. Scale bar, 50 μm.

Figure 20. *Riftia pachyptila,* adult, TEM. Bacteriocyte in trophosome. Scale bar, 3 μm.

Figure 21. *Ridgeia* sp. Rank D-nr. #13, sagittal section, epon, 1.5 μm. Anterior of larva. Scale bar, 30 μm.

Figure 22. Rank H-nr. #15, sagittal section, epon, 1.5 μm. Anterior of larva. Arrows, ventral ciliated field. Scale bar, 30 μm.

Figure 23. Rank C-nr. #21, sagittal section, epon, 1.5 μm. Anterior of larva. Scale bar, 30 μm.

Figure 24. Rank CB7-nr. #20, frontal section, epon, 2.0 μm. Anterior of larva. Scale bar, 30 μm.

Figure 25. Rank CB1-nr. #23, transverse section, epon, 2.0 μm. Ventral ciliated field and adjacent paired ventral nerve cords. Large arrows, nerve cords; small arrows, extent of ventral ciliated field. Scale bar, 30 μm. BA, bacteria; BF, branchial filament; BR, brain; BV, axial blood vessel; CA, capillary; FG, foregut; HG, hindgut; LBF, larval branchial filament; MG, midgut; NU, nucleus; PB, presumed brain; PG, pyriform glands; VP, ventral process; VV, ventral vessel.

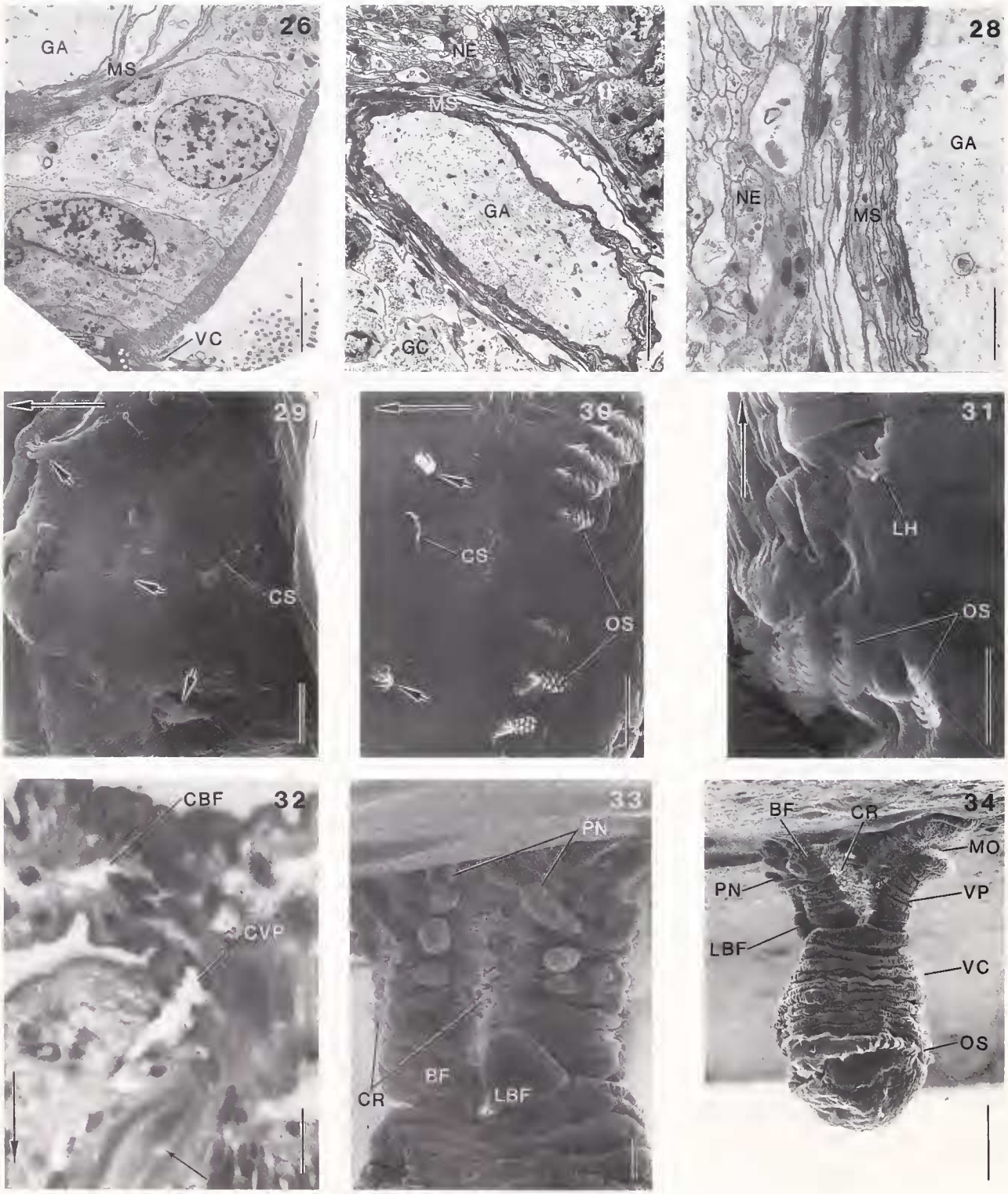


Figure 26. *Ridgeia* sp. Unranked juvenile, TEM. Cross-section of vestimentum in region of ventral ciliated field, showing portion of giant axon. Scale bar, 5 μm.

Figure 27. Unranked juvenile, TEM. Cross-section of nerve cord in vestimentum, showing giant axon, neurites and glial cell bodies of myelin sheath. Scale bar, 5 μm.

Figure 28. Unranked juvenile, TEM. Enlargement of myelin sheath of giant axon. Note varying thickness of lamellae of sheath. Scale bar, 1.5 μm.

plasm of the perikarya of the giant axons stains lightly in TEM preparations. Numerous mitochondria and dense-cored vesicles are visible in these cells. A single giant axon exits each perikaryon and extends ventrally through the neuropile of the brain. Microtubules, mitochondria, and dense-cored vesicles are present in the giant axons in this region.

Upon reaching the ventral ciliated field, the nerve cord diverges and carries one giant axon in each branch (Figs. 25, large arrows; 26, 27, GA). The cytoplasm of the giant axons in this region stains lightly in TEM preparations and contains scattered vesicles and mitochondria (Figs. 27, 29). Microtubules, however, have not been observed here. In the region of the vestimentum, each giant axon is surrounded by a myelin sheath of irregularly spaced lamellae (Figs. 26, 27, MS) derived from glial cells whose cell bodies are mostly clustered in the ventrolateral region of each nerve cord (Fig. 27, GC). Occasionally, a glial cell body is observed in the outermost lamellae of the sheaths. In one juvenile, the thickness of the sheaths varied from 0.5 μm to 2.5 μm, and each sheath consisted of about 12 lamellae, although it was difficult to determine this number precisely because the lamellae frequently branch and fold back on themselves (Fig. 28). By contrast, the number of lamellae surrounding the giant axon in the adult stage of *Riftia pachyptila* exceeds 50 (pers. obs., SLG). The layer of cytoplasm between membranes of the lamellae varies in thickness from as little as 50 nm to as much as 2 μm. Mitochondria, endoplasmic reticulum, and numerous vesicles are present in the cytoplasm of the thicker lamellae.

The nerve cords fuse at the posterior margin of the ventral ciliated field, and a single nerve cord extends through the trunk and opisthosome. In the trunk, the nerve cord contains one giant axon whose organization is similar to that of the giant axons in the vestimentum. The nerve cord in the opisthosome lacks a giant axon (Jones, 1981a, 1985a).

Further development of larvae and juveniles

If the 38 specimens examined by SEM are arranged from trochophore (Fig. 2) to established juvenile (Fig. 1), lengths from 58 μm to 4.04 mm, it is possible to determine the progressive development of a number of morphological characters (Table I; Figs. 29–52).

Larval setae. In the single trochophore observed, three larval hooks and one capillary seta are visible in one micrograph (Fig. 29, small arrows, CS), and one other hook and two other capillary setae can be seen in micrographs taken at right angles to the first; about one-half of the circumference of the trochophore, at the level of the larval setae, is obscured by mounting adhesive. We suggest that this trochophore may bear up to eight larval hooks and six to eight capillary setae. In later stages, where observed, there are two pairs of larval hooks, situated laterally, with a single, capillary seta between each pair (Fig. 30, small arrows, CS; Jones and Gardiner, 1988, Fig. 7E). Each hook bears a single cluster of three to five denticles, palmately arranged around a central one, all pointing anteriorly (Figs. 29–31, LH/small arrows). In longitudinal sections, it has been confirmed that the larval setae are situated in the trunk wall and are well-separated from the opisthosome and the opisthosomal setae (Fig. 50, LH). Larval setae persist for a longer or shorter time, apparently depending on the amount of abrasion in life or the amount of handling that the specimens receive during sorting and processing; capillary setae are lost even more readily. Figure 53 shows the presence or absence of larval setae among the ranked specimens and indicates that they are present on an individual as long as 1.74 mm.

Branchial filaments. The first (larval) branchial filaments develop as a pair on the dorsal surface of the larva, arising just posterior to the prototroch (Figs. 3, 4, 33, 34, LBF), and a second pair may develop just posterior to the first. These pairs of larval branchial filaments do not develop pinnules but bear two longitudinal rows of cilia, one dorsomedial and the other ventrolateral; both pairs are considered to be larval structures because they appear to be resorbed as development proceeds. In the youngest stage that was sectioned (G-nr. #4) there is a suggestion that the coelomic cavity of the ventral process (Fig. 32, CVP), at some time in development, communicates with the cavity of the larval branchial filaments (Fig. 32, CBF); the coelomic cavity of the ventral process was not

Figure 29. Rank #1, SEM. Larval setae on posterior of trochophore larva. Large arrow, anterior; small arrows, larval hooks. Scale bar, 5 μm.

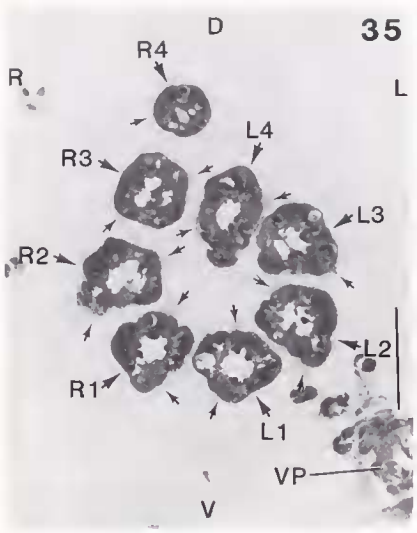
Figure 30. Rank nr. #21, SEM. Setae on posterior of larva. Large arrow, anterior; small arrows, larval hooks. Scale bar, 10 μm.

Figure 31. Rank #15, SEM. Setae on posterior of larva. Arrow, anterior. Scale bar, 10 μm.

Figure 32. Rank G-nr. #4, sagittal section, epon, 1.5 μm. Base of branchial filament and ventral process. Small arrow, mouth/foregut; large arrow, ventral. Scale bar, 10 μm.

Figure 33. Rank #10, SEM. Dorsal view of anterior part of larva. Scale bar, 10 μm.

Figure 34. Rank #10, SEM. Left lateral view of larva. Scale bar, 50 μm. BF, branchial filament; CBF, coelom of branchial filament; CR, ciliary row(s); CS, capillary seta; CVP, coelom of ventral process; GA, giant axon; GC, glial cell bodies; LBF, larval branchial filament; LH, larval hook; MO, mouth; MS, myelin sheath; NE, neurites; OS, opisthosomal setae; PN, pinnule(s); VC, ventral ciliated field; VP, ventral process.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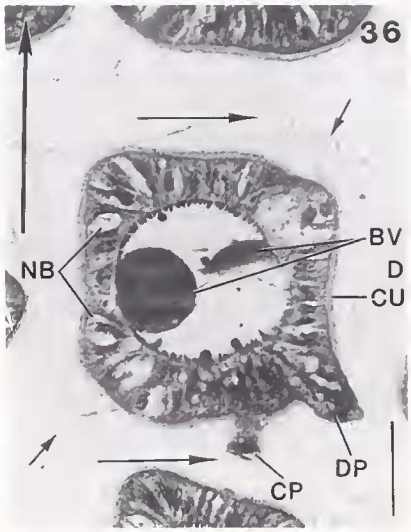
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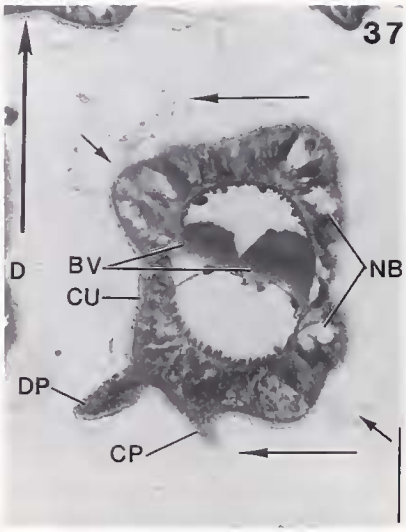
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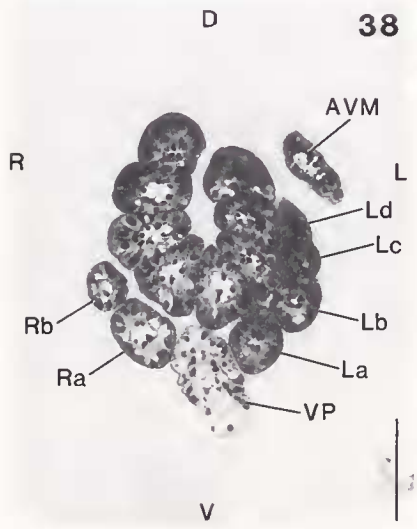
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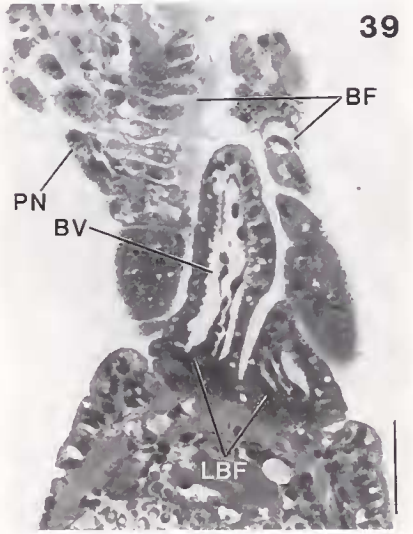
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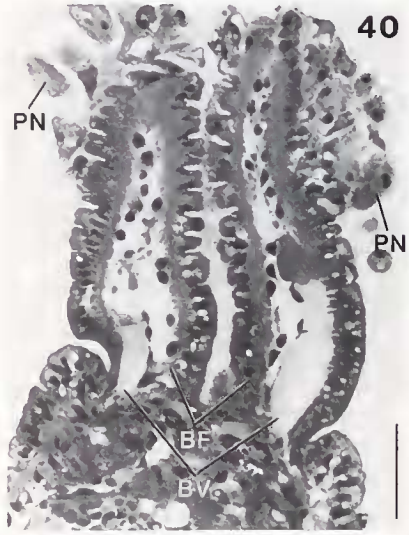
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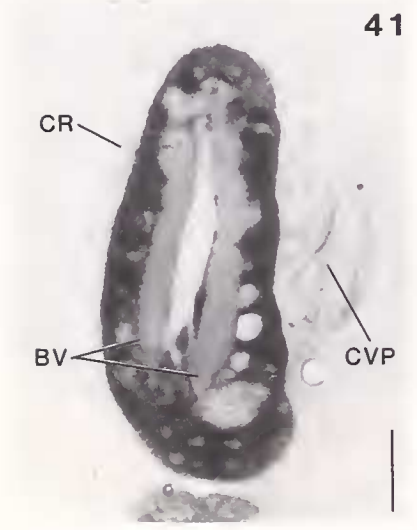
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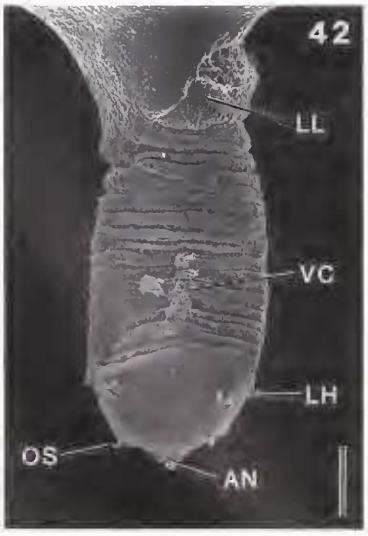
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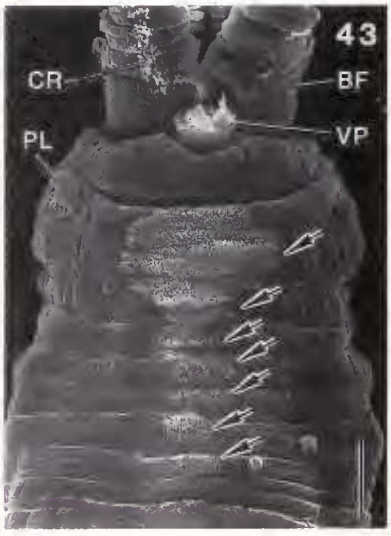
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observed in sections of other, older, larvae. Subsequent juvenile branchial filaments develop after formation of the ventral process and mouth and, initially, are provided with two rows of pinnules on their dorsal surface and ciliary tracts on their dorsomedial and ventrolateral surfaces (Figs. 33, 34, PN, CR). The second, as well as subsequent, juvenile branchial filaments initially develop with the same disposition of pinnules and ciliary tracts as the first filaments (Figs. 8, 51, PN, CR). At about the time of appearance of the second pair of juvenile branchial filaments, the first pair of branchial filaments appears to undergo a reorientation of pinnules, which become more lateral (Fig. 8, PN), and of ventrolateral ciliary tracts, which become more ventral (Fig. 43, CR). The reorientation continues until, at a point where four pairs of branchial filaments are present in the first branchial lamella, pinnules are situated on the outer faces of filaments (Fig. 35, large arrows) and ciliary tracts are situated adjacent to the rows of pinnules, ventral or lateral, and on the surface of the filaments, directly opposite each other (Fig. 35, small arrows). This disposition of rows of cilia is continued throughout the life of the worm (Figs. 36, 37, small arrows). In adults, two rows of pinnules are on the outer face of the filament, one row is central and the other is displaced to the outer dorsal area (Figs. 36, 37, CP, DP). It may also be noted that each filament carries two bundles of nerves just below the ventral-facing surface, that the pair of branchial blood vessels is in line with the axis of the lamella, that the cuticle, generally rather thick, is quite thin over the ciliated cells and over the surface of the pinnules (Figs. 36, 39, NB, BV, CU), and that right branchial filaments are mirror images of left filaments (Figs. 35–37).

Branchial filaments are added in paired rows (Figs. 35, 38) that are the forerunners of the branchial lamellae of older specimens. The oldest filaments of a lamellar series are ventral and medial, adjacent and lateral to the ventral process or dorsal to it (Figs. 9, DBF; 35, R1, L1; 38, Ra, La), and younger filaments are added laterally and dorsally (Figs. 35, R4, L4; 38, Rb, Ld). Subsequent lamellae develop in the same manner, outside the previous lamellae (Figs. 38, 46). Relative to later-developing branchial filaments, larval filaments appear to lack well-developed longitudinal muscles, as well as pinnules (Figs. 39, 40). In earlier stages (#'s 3–9), larval branchial filaments are the primary respiratory surface for developing *Ridgeia* and are so served by simple vascular loops (Fig. 41). Later, respiratory surfaces are enhanced by the development of elongated filaments bearing pinnules (Fig. 40). Figure 54 discloses that, after they appear, there is a modest increase in the number of branchial filaments, to about eight, until a length of about 0.5 mm, after which the number of filaments is great enough to preclude their being counted.

Ventral ciliated field. There is no neurotroch on the trochophore. Up to nine tufts of cilia, linearly arranged and, perhaps, representing a late-developing neurotroch (from #3 on), are present on the mid-ventral surface of

Figure 35. *Ridgeia* sp. CB1-nr. #23, transverse section, epon, 2.0 μm. Cross-section, four pairs of branchial filaments. L1 (oldest)—L4 (youngest), left filaments; R1 (oldest)—R4 (youngest), right filaments; large arrows, general location of pinnules; small arrows, location of ciliary rows. Scale bar, 50 μm.

Figure 36. *Ridgeia piscesae*, USNM No. 98106, adult, transverse section, paraffin, 5 μm. Cross-section, left branchial filament. Large/thick arrow, direction to longitudinal axis of worm; long/thin arrows, direction of effective stroke of ciliary rows, *Riftia pachyptila*; short arrows, location of ciliary rows. Scale bar, 30 μm.

Figure 37. *Ridgeia piscesae*, USNM No. 98106, adult, transverse section, paraffin, 5 μm. Cross-section, right branchial filament. Large/thick arrow, direction to longitudinal axis of worm; long/thin arrows, direction of effective stroke of ciliary rows, *Riftia pachyptila*; short arrows, location of ciliary rows. Scale bar, 30 μm.

Figure 38. *Ridgeia* sp. CB1-nr. #23, transverse section, epon, 2.0 μm. Cross-section, base of branchial plume, two pairs of branchial lamellae (proximal to Fig. 35). La (oldest)—Ld (youngest) filaments of second left lamella; Ra (older)—Rb (younger) filaments of second right lamella. Scale bar, 50 μm.

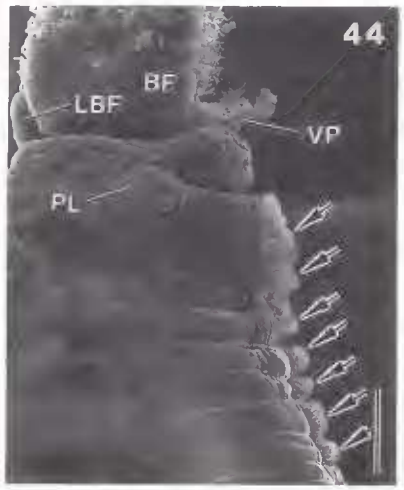
Figure 39. Rank CB7-nr. #20, frontal section, epon, 2.0 μm. Base of larval branchial filaments. Scale bar, 30 μm.

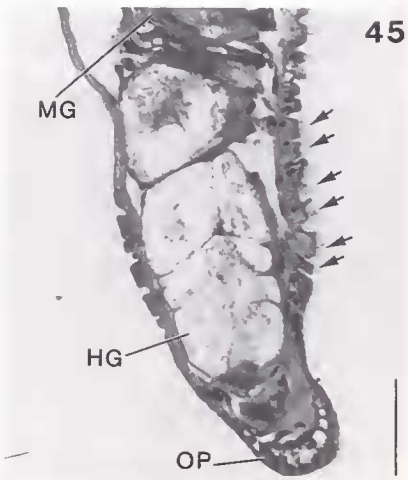
Figure 40. Rank CB7-nr. #20, frontal section, epon, 2.0 μm. Base of branchial filaments. Scale bar, 30 μm.

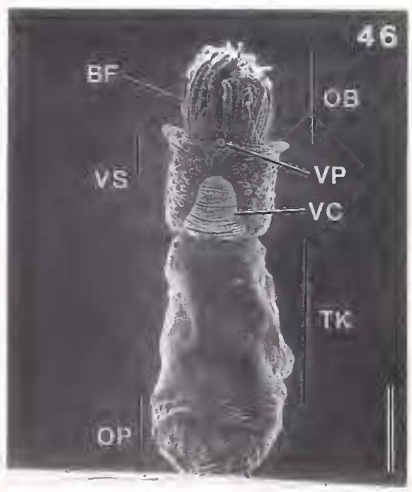
Figure 41. Rank F-nr. #10, longitudinal section, epon, 1.5 μm. Larval branchial filament, showing vascular loop. Scale bar, 10 μm.

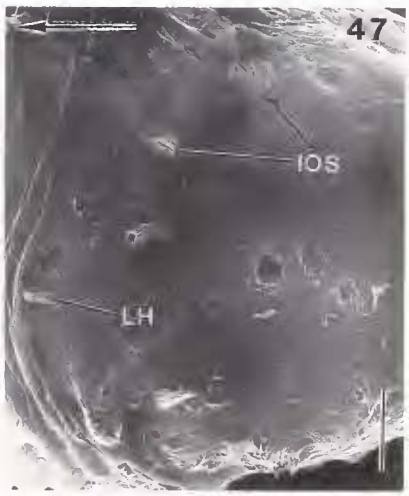
Figure 42. Rank #9, SEM. Ventral view showing posterior position of segmented ventral ciliated field. Scale bar, 30 μm.

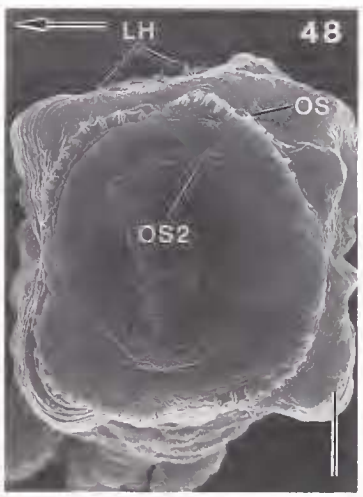
Figure 43. Rank #20, SEM. Ventral view, showing segmented ventral ciliated field (arrows); ventral process damaged. Scale bar, 30 μm. AN, anus; AVM, anterior vestimental margin; BF, branchial filament(s); BV, blood vessel(s); CP, central pinnule; CR, ciliary row; CU, cuticle; CVP, cilia of ventral process; D, dorsal; DP, dorsal pinnule; L, left; LBF, larval branchial filaments; LH, larval hook; LL, lower lip; NB, nerve bundles; OS, opisthosomal seta; PL, vestimental plaque; PN, pinnule; R, right; V, ventral; VC, ventral ciliated field; VP, ventral process.

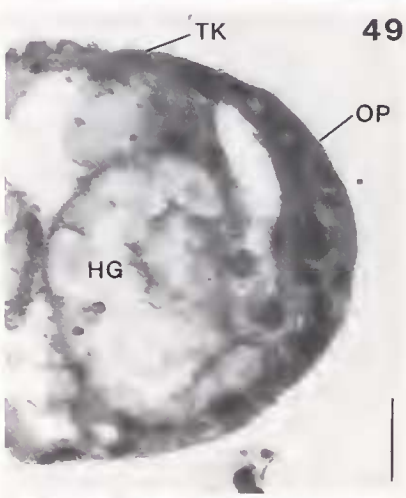


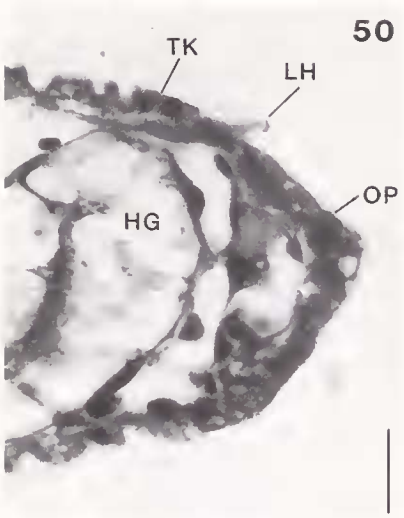


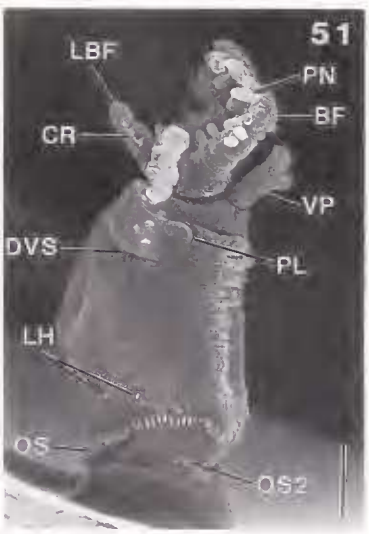












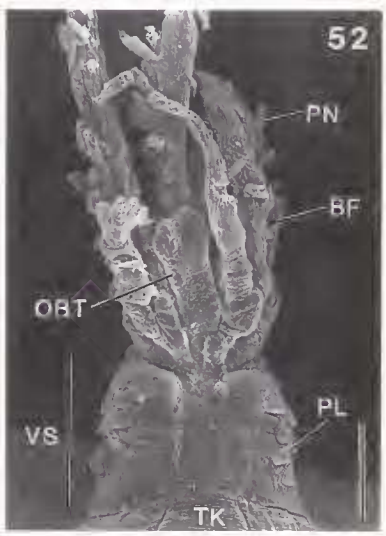


Figure 44. *Rudgeia* sp. Rank #20, SEM. Right lateral view, showing segmented ventral ciliated field (arrows); ventral process damaged. Scale bar, 30 μm.

Figure 45. Rank D-nr. #13, sagittal section, epon, 1.5 μm. Segmented ventral ciliated field (arrows). Scale bar, 30 μm.

Figure 46. Rank #33, SEM. Ventral view, showing fused ventral ciliated field. Scale bar, 30 μm.

the posterior one-half to one-third of the vestimentum-trunk of larvae and young juveniles (Fig. 42, VC); these tufts ultimately fuse to form the pear-shaped ventral ciliated field of adults (Fig. 1, VC). Although the tufts present the appearance of being segmentally arranged (Figs. 43, 44, arrows), sagittal sections through the tufts and the body wall do not reveal any indication of internal segmentation (Fig. 45, arrows). In early stages, the tufts overlie the trunk cavity and are displaced anteriorly by differential growth of the posterior trunk region. By the time the vestimentum is formed, the ciliated tufts, laterally expanded, are fused to form the ventral ciliated field (Fig. 46, VC). Figure 55 reveals that the linearly segmented ventral ciliated field persists in specimens of up to about 0.5 mm in length and the pear-shaped field of adults is present in juveniles of greater length.

Opisthosomal setae. These are confined to the anterior opisthosomal segments. As in adults, they bear two groups of denticles, the larger group projecting anteriorly and the smaller, posteriorly (Figs. 30, 31, OS; see also Jones, 1985c, Figs. 15, 48, 52). The first opisthosomal setae of juveniles may bear a superficial resemblance to larval setae and have a single set of denticles (Fig. 47, IOS). Opisthosomal setae develop in transverse rows in the four quadrants of the body (Fig. 48, OS), and those of each side meet laterally; setal rows do not appear to meet dorsally or ventrally. Semi-thin sections of the posterior end of an early larva show that, prior to the appearance of the first row of opisthosomal setae, the opisthosome is undeveloped (Fig. 49, G-nr. #4). At the time of development of the first opisthosomal setae, internal segmentation has commenced (Fig. 50, F-nr. #10). Figure 56 shows that, until juveniles are about 1.0 mm long, there are usually up to four rows of opisthosomal setae, and it is only when a length of about 2.0 mm has been attained that the number of rows of setae begins to increase rapidly; there is a quite apparent variability in the development of setal rows (see Discussion, below).

Vestimentum. Development of the vestimentum is visible externally by the delineation of a pair of dorsolateral longitudinal ridges just posterior to the branchial filaments (Fig. 51, DVS). Further development leads to an extension of the posterior ends of these ridges, laterally and ventrally, to the posterior edge of the ventral ciliated field (Fig. 46). The presence of plaques also indicates the establishment of the vestimentum (Figs. 9, 43, 44, 51, PL). Much later (#36, >2.00 mm), a flange-like posterior vestimental margin, free of the trunk, is developed (Fig. 1, arrow). Figure 57 indicates that the vestimentum is established when juveniles are 0.44 mm in length and it may be developing from a juvenile-length of 0.18 mm.

Obturaculum. The developing obturaculum has not been observed among these larval and juvenile *Ridgeia.* Most probably its development is similar to that of *Riftia* (Fig. 52, OBT) where paired, medially fused, thin lobes, each about the diameter of a branchial filament, develop on the mid-line, dorsal to all branchial filaments. Figure 58 shows that the obturaculum of *Ridgeia* is not present up to a juvenile length of about 0.5 mm; its development may be initiated during the next 0.5 mm of growth. In the case of lengths greater than 1.0 mm, the obturaculum is present to support the developing branchial filaments.

Discussion

Trochophore

The discovery of a trochophore larval stage in the life history of a vestimentiferan provides an explanation of the geographical distribution of certain species of these worms. Vent fields may occur at 100–200 km intervals (Crane, 1985). Hydrothermal vents, to which most vestimentiferan species appear to be restricted, are discrete environments that, in the same general hydrothermal field, may be as close as 3 km to one another (on the Galapagos Rift: Rose Garden site to Mussel Bed site, about 10 km; Mussel Bed to Garden of Eden site, about 3 km) (J. F. Grassle, 1986) or as far apart as the Galapagos Rift and 13°N on the East Pacific Rise (EPR) (about

Figure 47. Rank #17, SEM. Lateral view, trunk/opisthosomal region showing profile of initial opisthosomal seta. Arrow, anterior. Scale bar, 10 μm.

Figure 48. Rank nr. #21, SEM. Posterior view of opisthosome, showing disposition of four sectors of opisthosomal setae. Arrow, ventral. Scale bar, 30 μm.

Figure 49. Rank G-nr. #4, sagittal section, epon, 1.5 μm. Section through undeveloped opisthosome. Scale bar, 10 μm.

Figure 50. Rank F-nr. #10, sagittal section, epon, 1.5 μm. Section through developing opisthosome. Scale bar, 10 μm.

Figure 51. Rank #16, SEM. Right lateral view, showing developing vestimentum. Scale bar, 50 μm.

Figure 52. *Riftia pachyptila,* SEM. Dorsal view, showing developing obturaculum. Scale bar, 100 μm. BF, branchial filament(s); CR, ciliary row; DVS, developing vestimentum; HG, hindgut; IOS, initial opisthosomal setae; LBF, larval branchial filament; LH, larval hook(s); MG, midgut; OB, obturacular region; OBT, obturaculum; OP, opisthosome or opisthosomal region; OS, opisthosomal setae, first row; OS2, opisthosomal setae, second row; PL, vestimental plaque; PN, pinnule; TK, trunk; VC, ventral ciliated field; VP, ventral process; VS, vestimentum.

2500 km farther northwest) and 21°N on the EPR (about 1100 km yet farther northwest), with *Riftia pachyptila* being common to all of these localities. The trochophore represents a life stage that can be transported by sea-bottom currents to maintain genetic continuity between widespread populations of a single species.

This particular specimen of *Ridgeia* (Fig. 2) was washed from among adult tubes and, apparently having settled, had not yet developed to the point where it was permanently in place, *i.e.*, in a tube affixed to a solid substratum. The development of larval setae (Fig. 29) suggests that this trochophore had developed sufficiently so that it was nearly ready to secrete a tube that would allow the proper functioning of the larval hooks in maintaining the larva within its tube.

If, as is thought, vestimentiferans fall into the Gastroneuralia, as used by Nielsen (1987), compound cilia are to be expected in the prototroch of *Ridgeia*. That they are not present may well be due to the incidental cavalier treatment received during fixation on shipboard, when the first thought was to preserve the whole sample, primarily adults. Nielsen (1987, p. 206) specifically states that a special, gentle preservation is necessary to maintain the integrity of compound cilia.

Although Southward (1988b) had no specimens smaller than nr. #5 and #6, she compared her smallest with the patterns of ciliation noted by Nielsen (1987) and suggested that the vestimentiferan larva belongs to the "trochophore type." We confirm her suggestion.

We were able to examine only one specimen that was young enough to be identified as a trochophore larva. Although this specimen lacks openings into the digestive system, as well as an apical ciliary tuft, neurotroch, metatroch, and telotroch, the complete ring of cilia situated near the apical end, which is the characteristic position and appearance of a prototroch, confirms the larva's identity as a trochophore. The presence of larval hooks suggests that it is a late trochophore and confirms that it is a larval vestimentiferan. The absence of digestive openings is possibly correlated with the young age of the specimen, whereas the absence of ciliary bands, other than the prototroch, could be related to age of the specimen or could reflect developmental features specific to vestimentiferans. The presence of a larva of the trochophore type in the early development of vestimentiferans provides additional evidence for the belief that vestimentiferans should be placed along the evolutionary line that includes annelids, molluscs and other smaller groups of protostomes.

Ventral process and gut

On the basis of our observations, we suggest that the prototroch and pretrochal region of the trochophore are intimately involved in the formation of the ventral process. The gut appears to develop at about the same time, but the anal opening may occur early or late in development, as reflected by length (Table 1).

The pathway of the foregut through the brain to the trophosome has been established (Jones and Gardiner, 1988; Southward, 1988b; Jones, 1988b); the ventral process has been reported to be present in juveniles of *Riftia* up to 15.5 mm in overall length but is not present in young adults of about 111 mm overall length (Jones and Gardiner, 1988). In the latter, and in still older specimens, the track of the foregut, now apparently closed and functionless, can be traced through the brain and vestimental musculature to the trophosome (pers. obs., MLJ).

TEM examination of cross-sections of the cilia of the lower lip indicates that the effective stroke of these cilia is inward (pers. obs., SLG), thus justifying our contention that the mouth, indeed, might be employed in picking up free-living bacteria and/or other particulate food material. Jones and Gardiner (1988) noted that cells of the foregut are multiciliated. Southward (1988b) suggested that, prior to the installation of symbiotic bacteria, *Ridgeia* larvae are ciliary feeders; she noted, in a small specimen (nr. #9), that bacteria were in the wall of the gut, ". . . apparently undergoing digestion . . ." and that rod-shaped bacteria were present in the opisthosomal hindgut. These observations suggest at least three possibilities concerning the intracellular bacteria: (1) if all bacteria are endosymbionts, then the digestion of some by the worm indicates the early use of the endosymbionts as a food source; (2) if all bacteria are not endosymbionts, then the digestive process has been initiated for some but not for others and would confirm an early phase of ciliary feeding by the larvae; and (3) if the undigested bacteria are endosymbionts and the digested bacteria are not endosymbionts, then a period of overlap occurs between the phase of ciliary feeding and the full establishment of the symbiotic association.

There are indications that the mouth/foregut, even though morphologically developed, may not be open until H-nr. #15, based on the presence of endosymbionts. This is supported by the fact that the midgut wall, in younger, sectioned individuals, is devoid of bacterial symbionts (Table I, Figs. 21, 45; Jones and Gardiner, 1988, Fig. 7C). Likewise, the anus, although present at #7 and #9, does not appear to open to the exterior until C-nr. #21, based on light microscopy. In a small individual (G-nr. #4) we have observed a presumptive mouth and a gut, but the anus is totally lacking.

Southward (1988b) stated that, based on sections, a mouth, complete gut, and anus were present in a two-filament-stage larva (RJ, nr. #9); no trophosome was, as yet, present (see *Delayed settlement*, below).

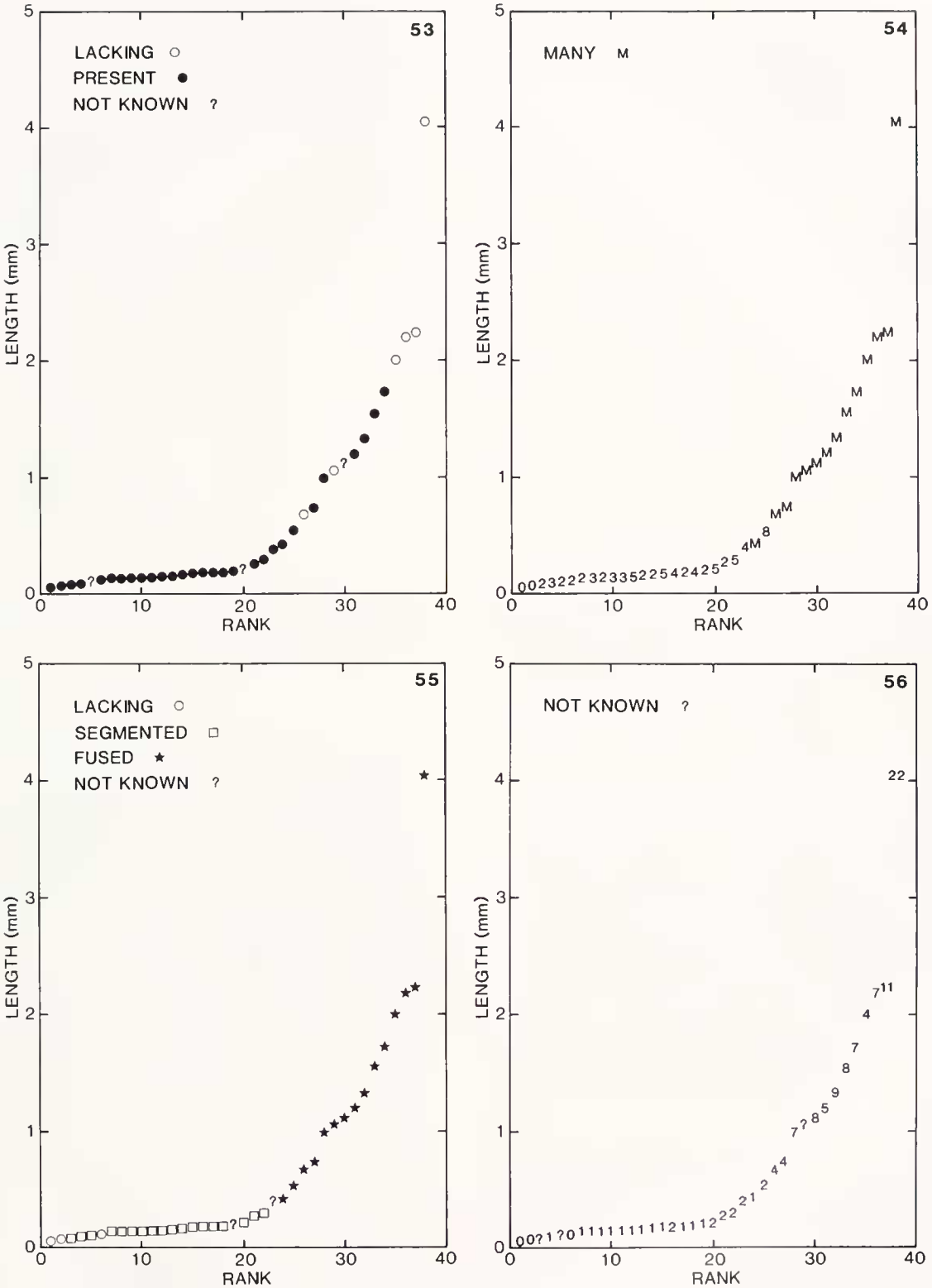


Figure 53. *Ridgeia* sp. Presence of larval setae among SEM specimens, ranked by length.
Figure 54. Numbers of branchial filaments among SEM specimens, ranked by length.
Figure 55. State of ventral ciliated field among SEM specimens, ranked by length.
Figure 56. Number of rows, opisthosomal setae, among SEM specimens, ranked by length.

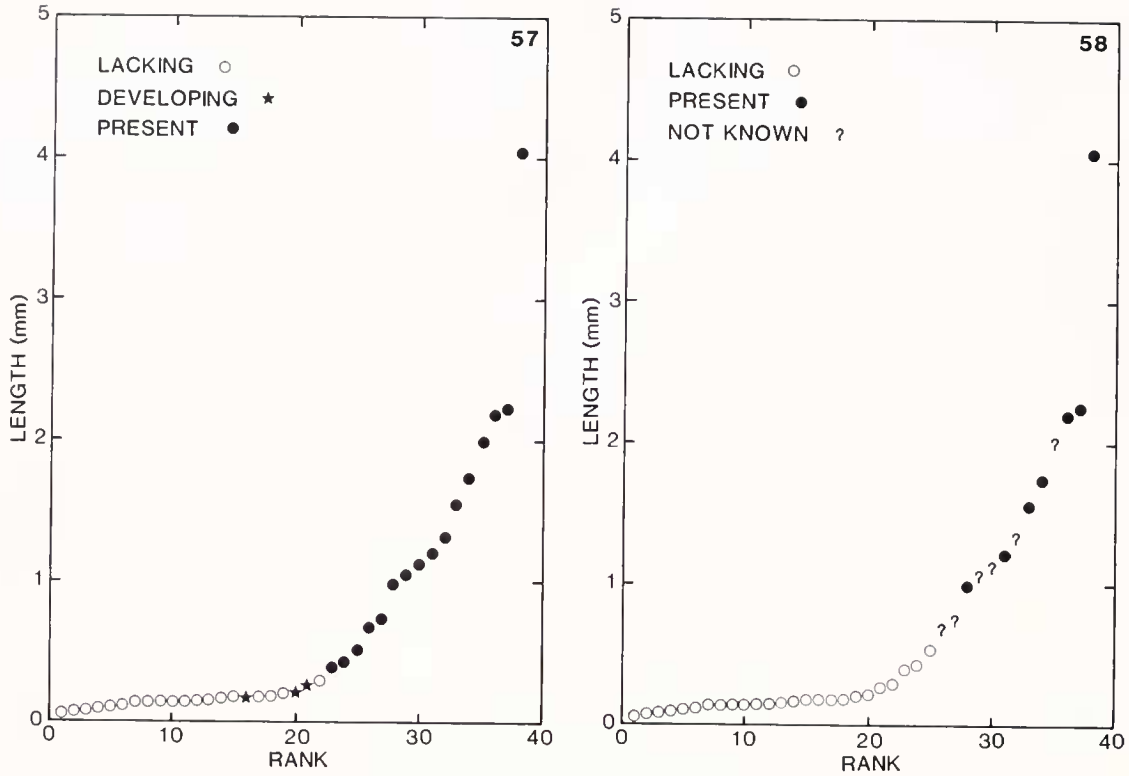


Figure 57. *Ridgeia* sp. Condition of vestimentum among SEM specimens, ranked by length.
Figure 58. Presence of obturaculum among SEM specimens, ranked by length.

Bacterial endosymbionts

Although we have not, as yet, reconstructed serial thin-sections and counted total numbers of bacteria in bacteriocytes, we have observed by TEM as many as 35 bacteria in a single section of a bacteriocyte. In this case, the bacteria occupied approximately 40% of the total area. Jones (1988b) elucidated details of the vascular system of the trophosome and observed that, in one instance, no bacterium was separated from a capillary by more than three bacteria. A chemoautotrophic mode of nutrition has been suggested for the bacteria in the trophosome of vestimentiferans (for extensive literature, see Jones and Bright, 1985, and Felbeck and Childress, 1988).

In previous accounts (Jones, 1988b; Jones and Gardiner, 1988), we suggested that the association between bacteria and the vestimentiferans *Riftia pachyptila, Oasisia alvinae,* and *Ridgeia* sp. begins with the ingestion of free-living bacteria, from surfaces available to the larvae/juveniles or from the waters surrounding the vent communities, into the transient digestive system of the worm. Once inside the gut, the appropriate bacterial strain, *i.e.,* sulfide-oxidizing, is phagocytized by midgut epithelial cells to establish the symbiotic association. Presumably other bacteria, non-essential to the vestimentiferan, not prospective endosymbionts, may be digested and used as a food source until the endosymbiotic association is established and the gut regresses.

Jannasch and Wirsen (1979) reported the presence of at least 200 different strains of free-living bacteria in vent waters and noted that most strains oxidized hydrogen sulfide or thiosulfate as their energy source. Oxidation of sulfide has been suggested as an energy source also for the endosymbiotic bacteria in *R. pachyptila* (see Arp *et al.,* 1985, for references). Distel *et al.* (1988) examined the 16S rRNA sequences of endosymbiotic bacteria from several invertebrate hosts, including *R. pachyptila.* Their data indicate that, in all cases, the host contains a single type of endosymbiont and hosts from different geographic localities possess similar types of endosymbionts. Based on these data, they concluded that the mechanism of selection of potential endosymbionts from the surrounding environment or the mechanism of transmittal of endosymbionts to new individuals must be specific. They suggested three possible mechanisms: (1) the symbiont is passed to the egg during spawning, (2) the symbiont is selected by the host during early development, and (3) the symbiont uses a host-specific "infection" (their quotation marks) mechanism.

At present, passing of endosymbionts to eggs during

spawning appears unlikely. Our TEM observations of eggs of *R. pachyptila* taken from just inside the aperture of the oviduct have not revealed the presence of endosymbionts in the egg cytoplasm or nuclei (pers. obs., SLG). TEM observations of freshly spawned eggs of *R. pachyptila* also have failed to reveal the presence of endosymbionts (Cary *et al.*, 1989). The method of establishment of the symbiotic association outlined by Jones (1988b) and Jones and Gardiner (1988) conforms to the second mechanism of Distel *et al.* (1988). However, this method also implies that midgut cells of larval/juvenile vestimentiferans are capable of distinguishing between potential endosymbionts and other, non-essential, bacteria; this method of recognition has not yet been determined.

We suggest the following possible sequence of events in the establishment of the symbiotic association: (1) engulfment of bacteria by the ventral process and their passage into the gut; (2) growth of one specific strain of bacteria close to midgut epithelial cells; and (3) phagocytosis of colonies of bacteria by midgut cells.

Nervous system

In his original description of *Lamellibrachia barhami*, Webb (1969) described the presence of a pair of tubes in the nerve cords in the vestimental region of that species. He indicated that these tubes ". . . enter the substance of the brain as very fine tubules" and that the nerve cord of the trunk contains a single tube throughout its length.

Van der Land and Nørrevang (1977) presented detailed descriptions of the organization and histology of fluid-filled "neurular tubes" in the nervous system of *Lamellibrachia luymesi*. They demonstrated that the arrangement of the tubes is similar to that described by Webb for *L. barhami*, except that a single tube extends only a short distance into the trunk region; the remaining nerve cord of the trunk lacks a neurular tube. They stated that, in the area of the brain, the tubes give off one or two side branches, which could correspond with Webb's observation. Because the opisthosome was not present on the specimens examined by Webb (see Jones, 1981a, p. 1310, 1985c, pp. 122–123) and by van der Land and Nørrevang, they could not comment on the organization of the nerve cord in that region. The wall of the tubes was described by van der Land and Nørrevang as "dense" and consisting of concentric fibers or lamellae in which nuclei are embedded.

Following the terminology of van der Land and Nørrevang (1977), Jones (1981a) described the organization of "neurular tubes" in *Riftia pachyptila*. In the vestimentum and trunk, the organization of the tubes reflects that of *L. barhami*. Because the opisthosome was present in Jones' specimens, he added the observation that the nerve cord in that region lacks a neurular tube.

Among invertebrates (excluding the "invertebrate chordate" groups), neural tubes of varying construction have been reported in the nervous systems of some Nemertea, Sipuncula, Echiura, and Hemichordata, whereas giant axons, which may present the morphological appearance of neural tubes in the light microscope, have been reported in some Nemertea, Phoronida, Sipuncula, Polychaeta, Oligochaeta, Cephalopoda, and Crustacea (for additional details and references, see Bullock and Horridge, 1965). Our TEM examination of the nerve cord of *Ridgeia* confirms the presence of paired giant axons in the paired nerve cords of the vestimentum and a single giant axon in the nerve cord of the trunk. We suggest that the nerve cords of *Lamellibrachia barhami* and *L. luymesi* be re-examined by TEM to confirm the presence of "tubes" or "neurular tubes," as reported by Webb (1969) and van der Land and Nørrevang (1977), respectively, or giant axons, as suggested by our study of *Ridgeia*. The description of the wall of the neurular tube in *L. luymesi* by van der Land and Nørrevang (1977) is consistent with the morphological appearance of the myelin sheath around the giant axons of *Ridgeia* and *R. pachyptila*, when viewed by TEM. This observation suggests that giant axons are present in *L. luymesi*. In the case of *Riftia pachyptila*, the nerve cord of the trunk has been examined by TEM (pers. obs., SLG) and the so-called "neurular tube" reported by Jones (1981a) is a giant axon surrounded by a myelin sheath.

Several alternatives may be suggested to explain the presence of two giant axons in the vestimentum and only a single axon in the trunk of *Ridgeia* and, presumably, all vestimentiferans. The single giant axon in the trunk may represent (1) the continuation of one of the giant axons that originate in the brain, (2) a third giant axon whose cell body is situated in the nerve cord near the border between the vestimentum and trunk, or (3) the product of the fusion of the two giant axons that originate in the brain. Fusion of giant axons has been reported in the nervous systems of other invertebrate groups, *e.g.*, cephalopods (Bullock and Horridge, 1965). In their description of the "neurular tubes" in *L. luymesi*, van der Land and Nørrevang (1977) included a reconstruction that shows the fusion of the tubes of the vestimentum to form the single tube of the trunk (near the posterior margin of the ventral ciliated field). Our preliminary examination of serial semi-thin sections (1.5 μm) of *Ridgeia* appears to support van der Land and Nørrevang's observation of fusion. However, additional light microscopic and TEM observations are required to confirm the exact nature of the organization of the giant axons in *Ridgeia*.

Giant axons are particularly well-developed among tube-dwelling animals, *e.g.*, the phoronids and the sabellid and serpulid polychaetes (Bullock and Horridge, 1965) and are frequently implicated as an important

component in the "startle response" of those animals, *e.g.*, the polychaete *Myxicola* (Nicol, 1948). A withdrawal response has been observed *in situ* in the case of *Riftia pachyptila* (pers. obs., MLJ). We suggest that the giant axons in vestimentiferan nervous systems have an important role in this response. In that regard, the myelin sheath observed around the giant axons of *Ridgeia* and *R. pachyptila* would enhance the speed of conduction of a nerve impulse in a manner analogous to the myelin sheath of vertebrate nerves (for additional discussion and references, see Jamieson, 1981).

Further development of larvae and juveniles

Of the characters of adult morphology followed in this study, the ventral ciliated field, vestimentum, and obturaculum are all established at a length (from prototroch or base of branchial filaments to posterior end) of 1.0 mm. There is a consistency of development of the ventral ciliated field from its "segmented" state to the fused field of the adult (Table I). Likewise, once the obturaculum appears during growth, it is always present, although it may be obscured by overlying branchial filaments. The vestimentum begins differentiation at a length of about 0.18 mm and is present, then, after a length of about 0.4 mm. There is a certain amount of variation in the initiation of vestimental development between specimens of 0.18 and 0.4 mm in length (Table I). Larval setae, having fulfilled their apparent function in providing an early holdfast for the developing larva, are of variable occurrence as they are worn, discarded, or extracted. The number of rows of opisthosomal setae remains approximately constant at one or two, until a length of about 0.5 mm, and then new rows are added with considerable variation among specimens (see *Delayed settlement*, below). Branchial filaments develop in much the same manner, except that the variability in addition occurs earlier in development, at a length of about 0.16 mm.

Delayed settlement

Of the speculations concerning the dispersion of the larvae of bivalved molluscs of the hydrothermal vents, there is a consensus that the mussel, *Bathymodiolus thermophilus*, has a pelagic (J. P. Grassle, 1985) or planktotrophic (Lutz *et al.*, 1980; Berg, 1985; Turner *et al.*, 1985; Lutz, 1988) stage that may be epipelagic (Berg, 1985) or demersal (Lutz *et al.*, 1980; Turner *et al.*, 1985). The possibility of delayed settlement of such planktotrophic larvae has been suggested by Lutz *et al.* (1980). Warén and Bouchet (1989) have concluded that gastropods of the hydrothermal vents have a lecithotrophic development and can delay settlement until the proper habitat is encountered. Van Dover *et al.* (1985) have suggested a planktotrophic stage for the crab *Bythograea thermydron* and the shrimp *Alvinocaris lusca* and mention the possibility of delayed settlement for these species.

Southward (1988b) proposed that, in the case of *Ridgeia* sp., there is early ciliary feeding in developing larvae or juveniles but did not indicate whether this would take place in a pelagic or a demersal phase. Cary *et al.* (1989) observed that spawned eggs of *Riftia pachyptila* have a positive buoyancy due, in large part, to a considerable amount of cytoplasmic lipids. They suggest a pelagic development in *Riftia*, either planktotrophic, based on the small size of eggs, or lecithotrophic, based on the lipid content of the eggs (about half of the cytoplasmic volume). They further suggest that, as development proceeds, buoyancy decreases and that, ultimately, the developing *Riftia* return to the sea floor and settle in response to cues noted by Lutz *et al.* (1980), in the case of *Bathymodiolus*.

The preceding observations bear on the following discussion.

Based upon the nine specimens sectioned (Table I), there is no apparent consistency in the presence of an anus or of internal bacteria, during development, as reflected by length. Specimens G-nr. #4 and E-nr. #16 lack an anus and bacteria; H-nr. #15 and A-nr. #21 have both an anus and bacteria; B-nr. #15 and C-nr. #21 have an anus but lack bacteria. These inconsistencies might have a reasonable explanation.

It may be assumed that a developing embryo of *Ridgeia* will be positively buoyant (Cary *et al.*, 1989). As a mouthless trochophore among the plankton, lecithotrophic development can proceed only so far and further development would be postponed until a vent site is encountered (Warén and Bouchet, 1989). If there is further development in the plankton, resulting in the formation of the ventral process and the mouth, a planktotrophic phase would be initiated (Berg, 1985; Turner *et al.*, 1985; Lutz, 1988), ciliary feeding would be possible (Southward, 1988b), and a later anal opening for the gut would establish an efficient, one-way digestive tract; along with this, there might be a reduction of larval buoyancy (Cary *et al.*, 1989). Upon finding a hydrothermal vent, using cues like those suggested by Lutz *et al.* (1980), and settling there, the developing larva would be able to acquire sulfide-oxidizing bacteria for its trophosome. Because the trophosome will become internally isolated, there would now be no necessity for a hindgut or an anus; these could be resorbed and, over time, the ventral process, the mouth, and the foregut could be resorbed or become atrophied. Under this scheme the larval branchial filaments would serve as relatively simple respiratory surfaces for an early larva still using its original supply of yolk or a later form, now feeding by cilia. The later branchial filaments, with their better-developed rows of cilia and the augmented respiratory surface afforded by

rows of pinnules, would serve as efficient structures for taking up, among other things, sulfide (for the endosymbiotic bacteria) and oxygen (for both the bacteria and the worm), both of which are crucial for the survival of the worm. If the developing larva does not quickly encounter a hydrothermal vent, then it can survive by maintaining ciliary feeding, grows more than it might otherwise and, when finally arrived at a vent site, proceeds with the acquisition of bacteria and the closing of the anal opening at a more advanced stage of development and at a length greater than its early-arriving neighbors. The following is suggested, in summary (Table I):

1. G-nr. #4, F-nr. #10, D-nr. #13, E-nr. #16, at the time of preservation, were still using yolk for nutrition or were feeding by cilia (no anus, no bacteria);
2. H-nr. #15 and A-nr. #21 had obtained endosymbiotic bacteria and the anus could atrophy (with anus, with bacteria);
3. B-nr. #15 and C-nr. #21 were still ciliary feeders, newly arrived at the vent site, not yet with endosymbionts (with anus, no bacteria).

Applying these summary statements, it might be that a larger size than expected, as in the case of D-nr. #13, A-nr. #21, and C-nr. #21 (relative to G-nr. #4, H-nr. #15, and B-nr. #15, respectively), suggests a delayed settlement. In addition, E-nr. #16, apart from size, has characters that might place it near #7 or #8 (Table I); D-nr. #13 (Fig. 21) and C-nr. #21 (Fig. 23) show about the same relative development of the ventral process; and #31 and #35 (Table I) have fewer rows of opisthosomal setae than might be expected; perhaps E-nr. #16, C-nr. #21, #31 and #35 all had a delayed settlement, as well. Further, #5 (Fig. 6) and #14 (Fig. 7) have about the same development of the ventral process, whereas #15 (Fig. 8), although of about the same length as #14, has a much better developed ventral process and its branchial filaments appear to be further along in development; this suggests that #14 might have had a delayed settlement. Of Southward's specimens RB (nr. #32) and RC (nr. #33), both about the same length (Southward, 1988b), RC agrees best with the ranked specimens near #33, as regards development of characters, and RB would fit better at about #25; perhaps RB had a delayed settlement. Finally, Southward's specimen RJ (nr. #9), with a mouth, gut (without endosymbionts), and anus, would appear to be further along in development than our F-nr. #10 and D-nr. #13, in both of which the anus has not yet appeared.

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